Factors affecting dispersal in a butterfly -

A comparison between core and edge populations

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ABSTRACT

Abstract

In the current era of anthropogenic climate change is the long-term survival of all organisms dependent on their ability to respond to changing environmental conditions either by (1) phenotypic plasticity, which allows species to tolerate novel conditions, (2) genetic adaptation, or (3) dispersal to more suitable habitats. The third option, dispersal, allows individuals to escape unfavorable conditions, the colonization of new areas (resulting in range shifts), and affects patterns of local adaptation. It is a complex process serving different functions and involving a variety of underlying mechanisms, but its multi-causality though has been fully appreciated in recent years only. Thus, the aim of this doctoral thesis was to disentangle the relative importance of the multiple factors relevant to dispersal in the copper butterfly *Lycaena tityrus,* including the individual condition (e.g. morphology, physiology, behavior) and the environmental context (e.g. habitat quality, weather). *L. tityrus* is a currently northward expanding species, which makes it particularly interesting to investigate traits underlying dispersal. In the first experiment, the influence of weather and sex on movement patterns under natural conditions was investigated. Using the Metatron, a unique experimental platform consisting of interconnected habitat patches, the second experiment aimed to examine the influence of environmental factors (resources, sun) on emigration propensity in experimental metapopulations. Human-induced global change (e.g. climate change, agricultural intensification) poses a substantial challenge to many herbivores due to a reduced availability or quality of feeding resources. Therefore, in the third experiment, the impact of larval and adult food stress on traits related to dispersal ability was investigated. Additionally, the effect of different ambient temperatures was tested. In the fourth experiment, core (Germany) and recently established edge (Estonia) populations were compared in order to explore variation in dispersal ability and life history traits indicative of local adaptation. Dispersal is often related to flight performance, and morphological and physiological traits, which was investigated in experiments 2-4. Butterflies were additionally subjected to behavioral experiments testing for the individual's exploratory behavior (experiments 3 and 4).

Males and females differed substantially in morphology, with males showing traits typically associated with a better flight performance, which most likely result from

selection on males for an increased flight ability to succeed in aerial combats with rivalling males and competition for females. This pattern could be verified by mobility measures under natural conditions and flight performance tests. Interestingly, although females showed traits associated with diminished flight performance, they had a higher emigration propensity than males (though in a context dependent manner). Reasons might be the capability of single mated females to found new populations, to spread their eggs over a wide range or to escape male harassment. Conditions indicative of poor habitat quality such as shade and a lack of resources promoted emigration propensity. The environmental context also affected condition and flight performance. The presence of resources increased the butterflies' condition and flight performance. Larval and adult food stress in turn diminished flight performance, despite some reallocation of somatic resources in favor of dispersal-related traits. These detrimental effects seem to be mainly caused by reductions in body mass and storage reserves. A similar pattern was found for exploratory behavior. Furthermore, higher temperatures increased flight performance and mobility in the field, demonstrating the strong dependence of flight, and thus likely dispersal, on environmental conditions. Flight performance and exploratory behavior were positively correlated, probably indicating the existence of a dispersal syndrome. The population comparison revealed several differences between edge and core populations indicative of local adaptation and an enhanced dispersal ability in edge populations. For instance, edge populations were characterized by shorter development times, smaller size, and a higher sensitivity to high temperatures, which seem to reflect adaptations to the cooler Estonian climate and a shorter vegetation period. Moreover, Estonian individuals had an enhanced exploratory behavior, which can be advantageous in all steps of the dispersal process and may have facilitated the current range expansion.

In summary, these findings may have important implications for dispersal in natural environments, which should be considered when trying to forecast future species distributions. First, dispersal in this butterfly seems to be a highly plastic, context-dependent trait triggered largely by habitat quality rather than by individual condition. This suggests that dispersal in *L. tityrus* is not random, but an active process. Second, fast development and an enhanced exploratory behavior seem to facilitate the current range expansion. But third, while deteriorating habitat conditions are expected to promote dispersal, they may at the same time impair flight ability (as well as exploratory

behavior) and thereby likely dispersal rates. For a complete understanding of a complex process such as dispersal, further research is required.

INTRODUCTION

1 Introduction

Anthropogenic climate change and options for organisms to respond

Human-made climate change entails global warming as well as temperature anomalies and extreme weather events. These changes in turn have a high impact via effects on the water cycle, which can result in drought periods or heavy rainfall and floods (Rahmstorf & Coumou, 2011; Hansen *et al.*, 2012). The consequences for ecosystems and biodiversity will be enormous. Global warming is already causing substantial changes in abundance and phenology of organisms (Parmesan & Yohe, 2003; Parmesan, 2006; Chown *et al.*, 2010). Next to the above climatic changes, many other human-induced impacts on the environment can be observed, including land conversion resulting in habitat loss and fragmentation, the usage of environmental toxins and fertilizers, burning of fossil fuels or species overharvesting (Hansen *et al.*, 2012). Thus, long-term survival of all organisms is dependent on their ability to respond to these changing environmental conditions either by (1) phenotypic plasticity, which allows species to tolerate novel conditions, (2) genetic adaptation, or (3) dispersal to more suitable habitats, often resulting in range shifts (Warren *et al.*, 2001; Davis *et al.*, 2005; Hickling *et al.*, 2006; Berg *et al.*, 2010; Hofmann & Todgham, 2010).

The first option, phenotypic plasticity, describes the ability of a genotype to produce different phenotypes depended on environmental conditions. Thus, it represents direct environmental effects on the phenotype (Bradshaw, 1965; Fischer & Karl, 2010). These short term responses allow for maximum flexibility and might be crucial for species survival in the current era of climate change (Franks & Hoffmann, 2012; Kellermann *et al.*, 2012). But plastic responses are also costly, the most common cost is the maintenance of the sensory and regulatory machinery needed for plasticity, which frequently require energy and material expenses (DeWitt *et al.*, 1998; Pigliucci, 2005). Second, in the long term, environmental variation may induce evolutionary (genetic) adaptation, which is considered to be less costly, but often requires stable environments to evolve (DeWitt *et al.*, 1998). Further, even rapid evolutionary changes might be too slow to keep up with quickly changing environmental conditions (Williams *et al.*, 2008; Merilä, 2012). This leads us towards the third option, dispersal, allowing organisms to move away from unsuitable areas in order to find a better habitat to settle

(e.g. Matthysen, 2012). Hence, understanding the ultimate and proximate causes of dispersal will help to improve population management and predicting consequences of environmental changes on species persistence and distributions (Cote *et al.*, 2010a).

In this thesis, the focus will be mainly on the last option, dispersal, but nevertheless is the survival and maintenance of species often determined by a combination of all three options. Four main studies were conducted, focusing on factors underlying and facilitating dispersal. As study organism the butterfly *Lycaena tityrus* was used as butterflies in general are excellent model organisms in spatial ecology, especially in dispersal studies (see below; Settele *et al.*, 2009; Stevens *et al.*, 2010a; Ducatez *et al.*, 2012).

Dispersal

Dispersal is of crucial importance in driving a species' capacity to respond to human-induced environmental changes like unpredictable climatic variation or habitat loss and fragmentation (Cote & Clobert, 2007; Travis *et al.*, 2013). It is often defined as any movements potentially leading to gene flow (Ronce, 2007) and a complex process serving different functions and involving a variety of underlying mechanisms (Bowler & Benton, 2005; Bonte *et al.*, 2012; Matthysen, 2012). Dispersal involves three consecutive phases, which are departure from the current patch, movement between the patches (transient phase), and settlement in a new patch (Bowler & Benton, 2005; Clobert *et al.*, 2009). The evolutionary causes of dispersal include the avoidance of kin competition, inbreeding, resource competition and environmental stochasticity (Bowler & Benton, 2005; Ronce, 2007; Clobert *et al.*, 2009). Dispersal can also incur costs, for instance energetic, time and opportunity costs (reviewed in Bonte *et al.*, 2012). Note that causes and costs might differ between the three stages of the dispersal process (Bowler & Benton, 2005; Clobert *et al.*, 2009; Bonte *et al.*, 2012). The dispersal of individuals influences their individual fitness, but has also consequences for population dynamics and genetics through metacommunity regulation, gene flow or speciation, as well as for species' distributions (Hanski & Gilpin, 1997; Hanski, 1999; Roff & Fairbairn, 2001; Clobert *et al.*, 2012; Baguette *et al.*, 2013). This might be of special importance for the rescue of declining populations or the recolonization of empty habitat patches

after local extinctions (Hanski & Gilpin, 1997; Hanski, 1999; Parmesan, 2006). Moreover, dispersal is a key process in the colonization of new areas to track shifting climate niches and thereby affecting patterns of local adaptation (Doebeli & Dieckmann, 2003; Hickling *et al.*, 2006; Ronce, 2007).

Quantifying dispersal and dispersal proxies

The importance of predicting effects of dispersal on population dynamics seems to be clear, but practical problems associated with the study of dispersal often hinder accurate conclusions. Thus, theoretical studies play a particularly relevant role (Bowler & Benton, 2005). However, these model assumptions often lack a great deal of realism (Travis & French, 2000) and do not incorporate the condition-dependence of dispersal strategies (e.g. Hanski, 1994; Murrell *et al.*, 2002). Other studies found that models frequently underestimate the rate of long-distance dispersal, probably because these events are difficult to document and consequently also the rate of spread is hard to predict (Hastings *et al.*, 2005; Clark *et al.*, 2017). Furthermore, populations at range margins are often influenced by strong ecological and evolutionary forces, which are not or less experienced by populations within the range core (Lindstrom *et al.*, 2013). The difficulty of quantifying dispersal also depends on the model species. In insects, dispersal is relatively easy to measure in wing-dimorphic species, which often produce dispersive morphs characterized by wings and flight muscles (Roff & Fairbairn, 2007). In contrast, in wing-monomorphic insects (like butterflies), which comprise the vast majority of insect species, quantifying dispersal is more challenging as dispersal can be a continuously varying trait (Saastamoinen *et al.*, 2010, 2012) and more subtle changes in adult flight morphology are observed (Hill *et al.*, 1999, 2011). Thus, a number of proxies such as flight performance, wing shape and size or thorax mass and ratio (see below) are commonly used. Although the link between proxies and dispersal in the field has been frequently demonstrated (Hill *et al.*, 1999; Thomas *et al.*, 2001; Niitepõld *et al.*, 2009; Stevens *et al.*, 2010), these proxies might sometimes potentially measure components of flight that also share different functions (Saastamoinen *et al.*, 2012). Consequently, generalizations should be concluded carefully, and the potentially important role of proxies to examine dispersal evolution should not be neglected.

Multi-causality of dispersal and dispersal syndromes

Traits potentially affecting dispersal may include morphology, ecology, physiology, behavior and overall condition (Bowler & Benton, 2005; Bonte & Saastamoinen, 2012; Stevens *et al.*, 2013; Therry *et al.*, 2014). Considering the various functions and mechanisms, the dispersal process is likely driven by a complex interaction between several factors rather than a single trait (Bowler & Benton, 2005; Matthysen, 2012; Legrand *et al.*, 2015). Furthermore, growing evidence indicates that dispersal is not a fixed but a highly plastic trait (Ronce, 2007; Clobert *et al.*, 2009; Clobert & Chaine, 2012). Organisms seem to be able to respond to environmental cues / conditions during all steps of the dispersal process (Bowler & Benton, 2005; Ronce, 2007). In a recent study of Legrand *et al.* (2015) dispersal propensity was linked to a wide range of factors, including direct (morphology and performance), interactive (sex ratio) and indirect / interactive effects (sex, habitat quality, weather) clearly demonstrating the importance of comprehensive approaches.

Morphology and physiology

In most insects and many other animals, dispersal is related to flight performance. Flight performance in turn is influenced by morphological differences (Arnold, 1983; Gilchrist, 1990; Berwaerts *et al.*, 2002), which could also influence dispersal and colonization success, for instance in butterflies (Hill *et al.*, 1999; Sekar, 2012). Traits that have been associated with flight ability include wing size, wing aspect ratio, body size, wing loading, thorax mass, thorax-abdomen ratio, storage reserves, and flight metabolic rate (Betts & Wootton, 1988; Berwaerts *et al.*, 2002; Niitepõld *et al.*, 2009). For instance, high body mass may reflect an overall good condition, which can positively influence dispersal (Jenkins *et al.*, 2007). However, evidence exists for both larger and smaller individuals dispersing into new areas (reviewed in Bowler & Benton, 2005). A high body mass in combination with small wings leads to a high wing loading, which is likely detrimental for flight performance due to increased energetic costs (Wickman, 2009; Saastamoinen *et al.*, 2012). Almbro & Kullberg (2012) could demonstrate that a high wing loading reduced flight speed in male and led to a lower take-off angle in female *Pieris napi* butterflies. Nevertheless, high wing loadings have also been shown to be associated with fast flight (Betts & Wootton, 1988). Wing aspect

ratio, a trait that is related to the shape of the wing, is known to influence aerodynamic aspects of flight (Dudley, 2000; Hassall, 2015). High ratios have been shown to increase flight ability and acceleration capacity (Betts & Wootton, 1988; Berwaerts *et al.*, 2002; Berwaerts & Van Dyck, 2004). However, respective dispersal patterns are not consistent, and high as well as low ratios have been found to be associated with dispersing individuals (Hill *et al.*, 1999, 2011; Hassall, 2015). An important physiological trait associated with flight is fat, which represents the storage reserves of an organism. Fat is necessary to fuel flight activity and might thus also be a determinant of dispersal events (Zera *et al.*, 1998; Karl & Fischer, 2008). Note here that flight-related morphology / physiology could also show environmentally-induced plasticity, which may in turn feedback on dispersal ability (see below, environmental conditions). Many of the traits mentioned above are sex specific and likely result from differential selective pressures and thus causally underlie sexual differences in flight performance or dispersal strategies (Gilchrist, 1990; Van Dyck & Wiklund, 2002; Merckx & Van Dyck, 2005; Berwaerts *et al.*, 2006). Sex-biased dispersal has been shown in both directions, with males or females emigrating more frequently (Baguette *et al.*, 1998; Legrand *et al.*, 2015).

Behavioral and social factors

Factors affecting dispersal may also include behavioral traits such as exploration, aggression, and sociability (Dingemanse *et al.*, 2003; Duckworth & Badyaev, 2007; Cote *et al.*, 2010b; Ducatez *et al.*, 2012). For instance, inter-individual differences in exploration behavior have been linked to variation in dispersal propensity (reviewed in Cote *et al.*, 2010a) with explorative individuals dispersing more likely (Fraser *et al.*, 2001; Dingemanse *et al.*, 2003; Cote *et al.*, 2010a). Pronounced exploration skills could be advantageous in all steps of the dispersal process (Ronce, 2007; Cote *et al.*, 2010b), in making the decision to leave a habitat patch, facilitating the movement itself, and increasing the likelihood to find and successfully settle in a suitable habitat (Ducatez *et al.*, 2012). Conspecific density has also been suggested to influence dispersal activity (Dethier & MacArthur, 1964). Interestingly, both negatively and positively density-dependent dispersal in butterflies have been reported (Enfjäll & Leimar, 2005). Furthermore, conspecific density itself could affect the habitat quality in an area, for instance if the density exceeds the local carrying capacity and emigration

rates consequently increase (Dethier & MacArthur, 1964). However, as already highlighted above, single traits are unlikely to represent phenotypic differences. These differences often imply several traits, which could potentially result in syndromes.

Dispersal syndromes

Dispersal is an evolutionary and ecologically important process, but the motivation as well as the ability to disperse may differ strongly among populations and between individuals within populations (Van Dyck & Baguette, 2005; Baguette & Van Dyck, 2007; Ducatez *et al.*, 2014; Bestion *et al.*, 2015). In general, dispersing individuals do not seem to comprise a random subset of a given population. Rather, dispersers often possess several correlated life-history traits forming a so-called dispersal syndrome discriminating between dispersing and non-dispersing conspecifics (Bonte & Saastamoinen, 2012; Stevens *et al.*, 2013). Dispersal syndromes have been shown for a wide range of taxa (Fjerdingstad *et al.*, 2007; Moles & Westoby, 2014; Bestion *et al.*, 2015) and there is growing evidence for these syndromes in butterflies (Cote *et al.*, 2010a; Ducatez *et al.*, 2012; Saastamoinen *et al.*, 2012; Legrand *et al.*, 2015, 2016). In a recent study on *Pieris brassicae,* dispersing and resident individuals showed distinct behavioral and morphological attributes with good flight performers having longer wings and showing higher emigration rates (Legrand *et al.*, 2015). Further, flight performance in *P. brassicae* was also shown to be correlated with exploration skills (Ducatez *et al.*, 2012).

An individual's dispersal propensity also results from the interaction between the individual phenotype and local conditions (Clobert *et al.*, 2009). It seems that environmental conditions may have a considerable influence on traits related to dispersal (Saastamoinen *et al.*, 2012). Thus, dispersal syndromes are expected to show variation depending on environmental conditions (Ducatez *et al.*, 2014). Recent studies demonstrated that the environmental context might even prevail over phenotypic traits or social factors (e.g. Legrand *et al.,* 2015).

Environmental conditions

Dispersal is a crucial process to deal with environmental variation (Cote & Clobert, 2007; Travis *et al.*, 2013). Furthermore, organisms are expected to base their dispersal

decisions on informative cues, like environmental circumstances (Dall *et al.*, 2005; Clobert *et al.*, 2009), which has rarely been tested so far (but see Cote & Clobert, 2007; Baguette *et al.*, 2011). Conditions that have been found to influence dispersal include habitat quality (e.g. resource availability) and weather conditions (e.g. temperature, wind; Legrand *et al.*, 2015; Kuussaari *et al.*, 2016).

Temperature is one of the most important ecological factors, affecting species survival, performance and determining their distributions (Overgaard & Sørensen, 2008; Sunday *et al.*, 2011). Consequently, current increases in Earth's mean temperatures are expected to strongly affect ecosystems and biodiversity (Hansen *et al.*, 2012; Stanton *et al.*, 2015). Especially ectotherms are vulnerable to global temperature changes because their basic physiological functions such as locomotion, growth, and reproduction are strongly determined by ambient temperatures (Deutsch *et al.*, 2008). Hence, also insect flight is a temperature-dependent process, which may in turn directly influence dispersal rates (Delattre *et al.*, 2013; Kuussaari *et al.*, 2016). In general, butterflies require high muscle temperatures between 28-38 °C for flight activity, which is achieved through basking in the sun (Dennis, 1993; Watt, 1995; Wickman, 2009). Shady conditions will therefore substantially reduce overall flight activity. Indeed, dispersal rate was shown to decrease with increasing cloudiness (Cormont *et al.*, 2011; Kuussaari *et al.*, 2016). Wind speed and rainfall could also negatively influence dispersal (Kuussaari *et al.*, 2016).

Habitat quality can be influenced by the degree of fragmentation, which is expected to impose strong selective pressures on dispersal rates (Heino & Hanski, 2001; Schtickzelle *et al.*, 2006). Host plant abundance and abundance of nectar-providing flowers are also known to affect dispersal probabilities (Öckinger & Smith, 2008). For instance, predicted changes in temperature and precipitation and / or exploiting land-use practices could negatively influence the growth and phenology of plants, which may in turn negatively affect food availability or quality for herbivores (Parmesan & Yohe, 2003; Parmesan, 2006; Pleasants & Oberhauser, 2013; Lebeau *et al.*, 2016). The resulting food stress might negatively affect flight, which is highly energy demanding, due to widespread effects on body size, storage reserves and physiology (Candy *et al.*, 1997; Suarez, 2000).

Dispersal and associated traits are often affected by environmental conditions experienced immediately before and during dispersal (Bonte & Saastamoinen, 2012). But also environmental conditions experienced during development and even in previous generations may affect dispersal-related traits or adult dispersal behavior (Karlsson & Van Dyck, 2005; Bonte *et al.*, 2008; Bonte & Saastamoinen, 2012). For instance, suboptimal conditions (e.g. during development) may decrease the investment into costly dispersal traits (Bonte *et al.*, 2008). On the other hand, such conditions may increase dispersal ability or at least the willingness to do so, as an adaptive response for dealing with detrimental conditions in later life (e.g. Monaghan, 2008). This has been demonstrated through alterations in body composition or behavior (Bonte *et al.*, 2008; Saastamoinen *et al.*, 2010; van den Heuvel *et al.*, 2013).

Following these chapters, the complexity and multi-causality of dispersal seem apparently clear, highlighting the importance of comprehensive approaches, which are still rare. Thus, the aim of this work was to disentangle the relative importance of multiple factors relevant to dispersal from the individual phenotype through to environmental conditions. Moreover, to predict whether a species can shift to new areas requires additionally an understanding of the colonization process at the expanding edge of the species range.

Range shifts and local adaptation

The distribution of a species is determined by a suit of ecological and evolutionary processes (Gaston, 2003) and has evolved to maximize fitness in a particular ecological niche (MacArthur, 1972; Wingfield *et al.*, 2015). But recent environmental changes have impacted species habitats. At the southern range margins, we find environmental conditions shifting to warmer, unsuitable conditions, and corresponding range retractions or climate driven extinctions have become widespread (Parmesan *et al.*, 1999; Franco *et al.*, 2006; Thomas *et al.*, 2006). In contrast, at the northern margins conditions that were once too cold for species have now become suitable (Parmesan *et al.*, 1999; Parmesan, 2006; Bestion *et al.*, 2015). Considering rising temperatures, species could either adapt to new thermal conditions or they could track their shifting climate niche (see above, Parmesan, 2006). Several studies demonstrated poleward range shifts of species due to climate change (e.g. Parmesan *et al.*, 1999; Hickling *et*

al., 2006). Thomas (2010), for instance, estimated numbers of about 68% of all species. A study on nonmigratory European butterflies with data being obtained from northern and southern range margins could show that about 63% have shifted their distribution ranges to the north (by 35-240 km, Parmesan *et al.,* 1999). For instance, *L. tityrus* was frequently observed in the Montseny region of central Catalonia in the 1920s, but sightings from the 1990s are only from the Pyrenees, which is located 50 km to the north (Parmesan *et al.*, 1999). At the same time, *L. tityrus* entered Estonia for the first time and quickly established several successful breeding populations. Recent populations already stem from north-eastern Estonia (Õunap & Tartes, 2014).

A sufficient dispersal ability is only one aspect of range expansions. Once a suitable habitat has been reached by dispersing individuals, the ability to establish populations beyond the current range is of crucial importance. In 1967, Janzen stated a climatic variability hypothesis declaring that individuals at higher latitudes should have broader ranges of thermal tolerance. There is as well evidence for an increased flexibility in physiological traits at higher latitudes to cope with often more fluctuating environmental conditions (Janzen, 1967; Naya *et al.*, 2012). The variation of conditions along geographical gradients seems to require an ‘optimization’ of phenotypic values and consequently local adaptation (Hoffmann *et al.*, 2002; Castañeda *et al.*, 2005). Hence, phenotypic differences between recently established edge and long established core populations are expected (Hanski *et al.*, 2004; Calosi *et al.*, 2008; Lindstrom *et al.*, 2013), which might arise from differences in selection pressures but could also result from differences in the genetic structure of populations (Volis *et al.*, 1998; Purves, 2009; Mägi *et al.*, 2011). For instance, a positive relationship between thermal tolerance and latitude has been reported for different taxa (Calosi *et al.*, 2008; Naya *et al.*, 2012). Next to this, the process of range expansion itself could entail a strong selection pressure (Van Petegem *et al.*, 2016) Given that recent populations are founded by the most dispersive individuals (cf. Hill *et al.*, 1998; Hanski *et al.*, 2002, 2004) and dispersal-related traits are heritable (Roff & Fairbairn, 2001), more dispersive genotypes should accumulate at the expansion front (Phillips *et al.*, 2010; Shine *et al.*, 2011). Indeed, increased dispersal abilities at the range front have been demonstrated (e.g. Phillips *et al.*, 2006; Mitikka & Hanski, 2010; Van Petegem *et al.*, 2016).

Depending on dispersal ability, establishing capacity and thermal tolerance some species may benefit while others may suffer from current climate change (Pimm, 2001; Thomas *et al.*, 2001; Klockmann *et al.*, 2016). Many temperate zone ectotherms actually benefit from rising temperatures (Pimm, 2001; Deutsch *et al.*, 2008; Bestion *et al.*, 2015), so does the study organism, which is spreading and shifting its range northward.

Study organism

Lycaena tityrus (Poda, 1761), the sooty copper, is a butterfly of the Lycaenidae family (Fig. 1; Tolman & Lewington 1998). It is a widespread temperate-zone butterfly, ranging from Western Europe to central Asia. *L. tityrus* does not occur in Great Britain, Scandinavia and Siberia (Ebert & Rennwald, 1991). The species is bivoltine with two discrete generations per year in most parts of its range, although populations with one or three generations per year occur. Flight periods last from the end of April until June and from the beginning of July until September (Kolligs, 2014). Overwintering takes place as half-grown larva. The principal larval host-plant is *Rumex acetosa L.* (Polygonaceae), but several congeneric *Rumex* species are utilized as well (Ebert & Rennwald, 1991; Tolman & Lewington, 1998; Settele *et al.*, 2008). Adults are nectar feeders, using a broad array of species including several Asteraceae (Ebert & Rennwald, 1991). *L. tityrus* inhabits different habitat types, including unimproved grasslands, meadows, fenlands and forest edges (Ebert & Rennwald, 1991; Settele *et al.*, 2008). This species is currently expanding its range towards higher latitudes (see above) and altitudes, which is assumed to be largely driven by anthropogenic climate change (Brunzel *et al.*, 2008; Settele *et al.*, 2008; Klockmann *et al.*, 2016). For experiment 1 butterflies were individually tracked in the field. For the three following experiments (experiments 2-4) freshly eclosed, mated females from bivoltine populations were caught. Butterflies from the core of the range were sampled from three German populations and edge populations were sampled in north-eastern Estonia (Fig. 2). All females were transferred to climate chambers at Greifswald University for egg laying.

Figure 1 Top: *Lycaena tityrus* larvae (left side) and pupae (right side, http://www.bkmakro.de)
Middle: *L. tityrus* lateral view (left side: male © Lothar Feisel, right side: female © Jan Sevcik)
Below: *L. tityrus* top view (left side: male © Jens Philipp, right side: female © Robert Flogaus-Faust)

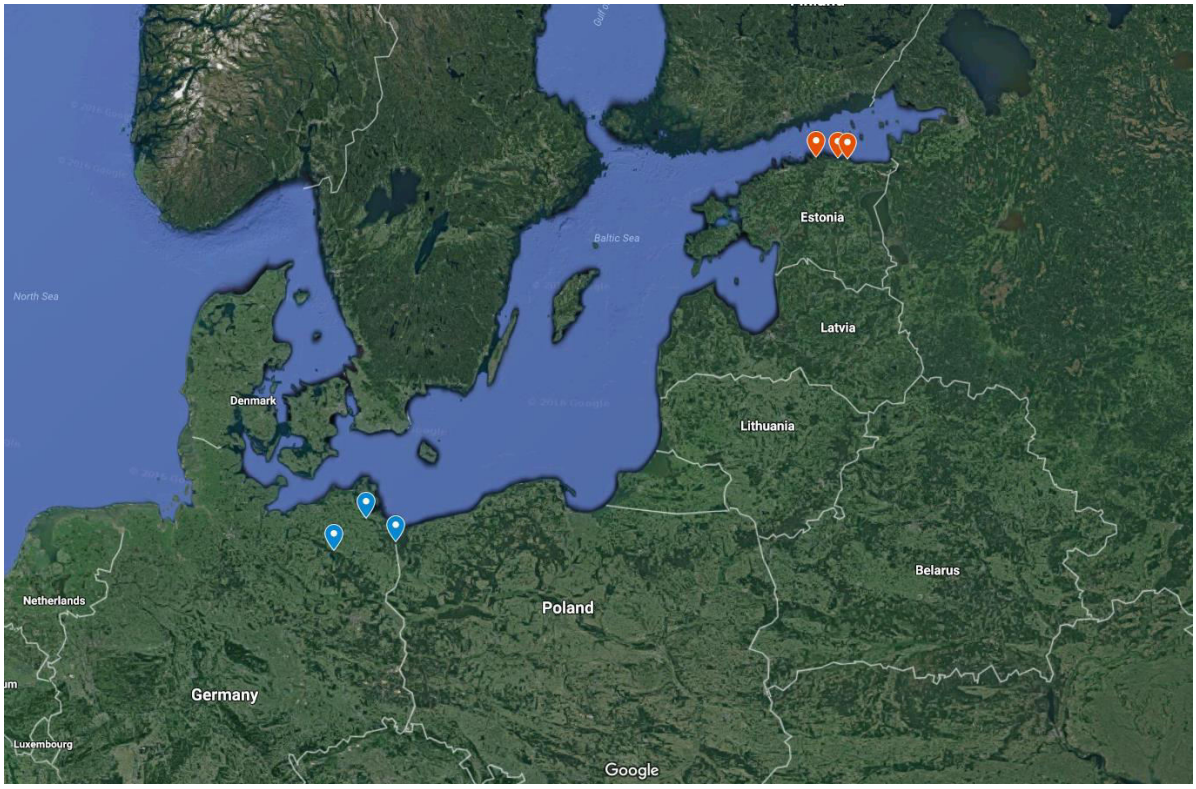

Figure 2 Sampling areas of *Lycaena tityrus* (map created with google maps)
Blue: German populations (Waren, Guest, Ueckermünde; f.l.t.r.)
Red: Estonian populations (Kasispea, Karepa, Mahu; f.l.t.r.)

SYNOPSIS

2 Synopsis

2.1 Influence of sex and weather on movement paths

Habitat loss and fragmentation as well as climate change are important threats to biodiversity conservation (e.g. Forester & Machlis, 1996; Sanderson *et al.*, 2002). The concomitantly deteriorating habitat quality often forces individuals to disperse to new habitats (e.g. Hanski, 1998). Thus, dispersal is important for dealing with environmental variation (Cote & Clobert, 2007; Travis *et al.*, 2013). However, dispersal ability may differ within and among populations (Van Dyck & Baguette, 2005; Baguette & Van Dyck, 2007; Ducatez *et al.*, 2014; Bestion *et al.*, 2015) and is dependent on environmental conditions such as prevailing weather conditions (Legrand *et al.*, 2015; Kuussaari *et al.*, 2016). Moreover, males and females may differ in their dispersal behavior (e.g. Fischer *et al.*, 1999).

Against this background, the study investigated sexual differences in and environmental effects on movement patterns in the copper butterfly *Lycaena tityrus.* Therefore, flight paths of both sexes were individually tracked (max. 15 min) within a habitat patch and weather conditions were recorded in parallel. As GPS devices Android smartphones in combination with a tracking application were used. Additionally, it was examined whether such devices are suitable for investigating insect behavior. The spatially restricted scale only allowed to examine movement behavior rather than dispersal per se, but addressing such questions could still provide valuable knowledge to assess dispersal abilities of populations or species (Niitepõld *et al.*, 2009). During tracking, several parameters (e.g. sex, number of stops) were recorded and the tracking app was used to extract data (e.g. total track length, average movement speed). To assess whether a high activity may facilitate dispersal, the linear distance between the starting and end point of each track was measured. Weather data were obtained from the weather station closest to the study area.

The study revealed sex-specific differences in behavioral patterns of *L. tityrus*. Males spent more time flying and had longer track lengths and linear distances than females. These differences might result from males' higher activity in general (see also Kingsolver, 1983), presumably owing to mate location and territorial disputes (Fig. 3a;

Ebert & Rennwald, 1991; Fischer & Fiedler, 2000). In addition, males alighted more often on flowers than females, probably to fuel their high flight activity (Fig. 3b). Females showed a higher affinity to host-plants for egg-laying (Fig. 3c). They seem to generally spend less time flying and allocate more of their time to oviposition (Kingsolver, 1983; Fischer *et al.*, 1999). Effects of environmental factors were less pronounced, but at least support the notion that detrimental weather conditions may negatively affect flight activity and therefore dispersal in insects (Cormont *et al.*, 2011; Kuussaari *et al.*, 2016).

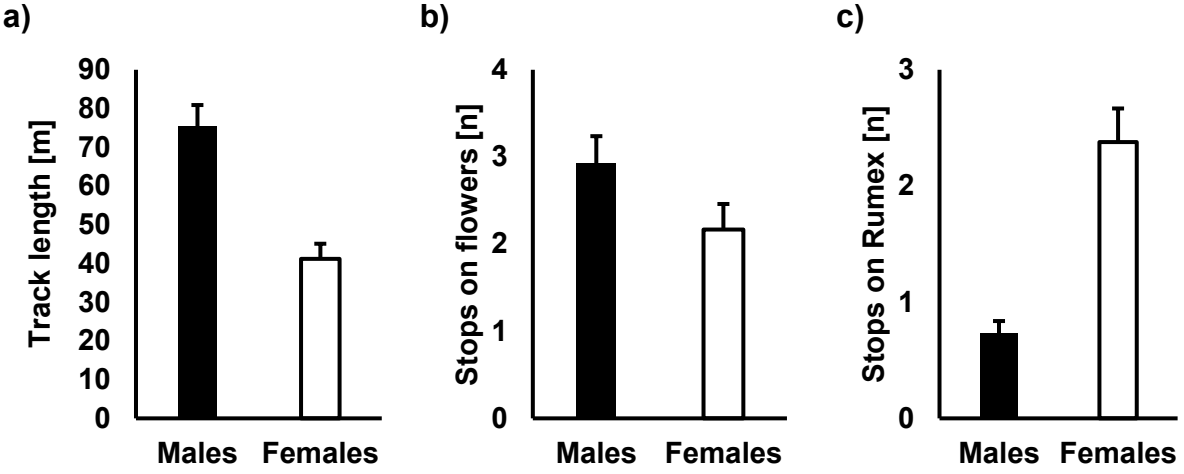

Figure 3 Sexual differences in *Lycaena tityrus* in track length (a) and the number of stops on flowers (b) and *Rumex* host-plants (c).

In summary, these findings provide a striking example of sex-related differences in animal behavior. Furthermore, customary smartphones can apparently comprise suitable means to reveal biologically significant behavioral patterns. More problematic than the technical device used seems to be the challenge of following individual butterflies for long enough in the field. Thus, the results seem to be not appropriate to estimate rates of dispersal and dispersal distances, such that any conclusions regarding insect dispersal need to be drawn with the greatest care.

2.2 Factors affecting emigration propensity and flight performance in a butterfly

Dispersal is a complex process serving different functions and involving a variety of underlying mechanisms (Bowler & Benton, 2005; Bonte *et al.*, 2012; Matthysen, 2012), but its multi-causality though has been fully appreciated in recent years only (reviewed by Matthysen, 2012). Currently, there is a lack of studies aiming to disentangle the relative importance of multiple factors relevant to dispersal, including the individual condition (e.g. morphology, behavior) and the environmental context (e.g. habitat quality, weather; but see e.g. Legrand *et al.*, 2015; Turlure *et al.*, 2016). Also, growing evidence indicates that dispersal is not a fixed but a highly plastic trait (e.g. Ronce, 2007) and organisms might be able to collect information on their current environment influencing their dispersal decision (Clobert *et al.*, 2009). However, only a few studies have focused on such cues triggering dispersal decisions (but see Cote & Clobert, 2007; Baguette *et al.*, 2011). These deficiencies are worrying given the crucial ecological and evolutionary importance of dispersal (Cote & Clobert, 2007; Clobert *et al.*, 2012; Travis *et al.*, 2013).

Against this background, a multifaceted approach was used to uncover the relative importance of several potentially dispersal-relevant factors in the butterfly *Lycaena tityrus*, ranging from the individual condition through to environmental variation. Using the Metatron, a unique experimental platform designed to study dispersal in experimental metapopulations (Legrand *et al.*, 2012), it was examined to what extent emigration propensity is affected by the environmental context. Therefore, two-patch experimental meta-populations were used where butterflies were released into departure cages with different environmental conditions (varied availability of sun and / or resources), in which they could either stay or which they could leave. Furthermore, flight performance was measured to examine relations between movement patterns under semi-natural conditions and flight performance under laboratory conditions (Ducatez *et al.*, 2012; Legrand *et al.*, 2015). In addition, individual variation in several morphological (e.g. wing size and shape, thorax mass and ratio, fat content) parameters was assed that may be directly or indirectly associated with movement ability (Legrand *et al.*, 2016).

The results show that males and females differed substantially in morphology. Males showed traits typically associated with a better flight performance, which most likely result from selection on males for an increased flight ability in order to succeed in aerial combats with rivalling males and in competition for females (Saastamoinen *et al.*, 2012). This could be verified in a flight performance test (Fig. 4). The environmental context affected condition and flight performance. The presence of resources in the departure cage increased the butterflies flight performance (Fig. 4).

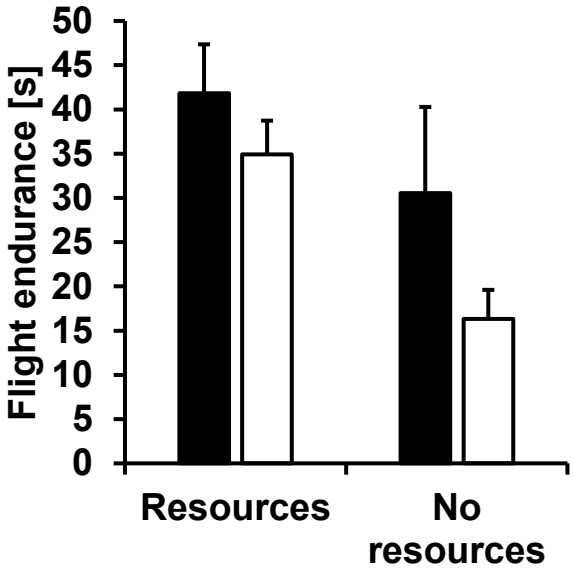

Figure 4 Flight performance (means + 1 SE) of *Lycaena tityrus* in relation to the presence or absence of resources in the departure cage. Males: filled bars; females: open bars

Interestingly, individual condition did not generally influence emigration propensity. Although females showed traits associated with diminished flight performance, they had a higher emigration propensity than males (when resources were present; Fig. 5). Reasons might be the capability of single mated females, in contrast to males, to found new populations or to escape male harassment (Hill *et al.*, 1999; Hopper, 1999; Trochet *et al.*, 2013). This indicates that flight performance and the first step of dispersal, i.e. the actual decision whether to disperse or not, do not necessarily equate. Moreover, conditions indicative of poor habitat quality such as shade and a lack of resources promoted emigration propensity (Fig. 5).

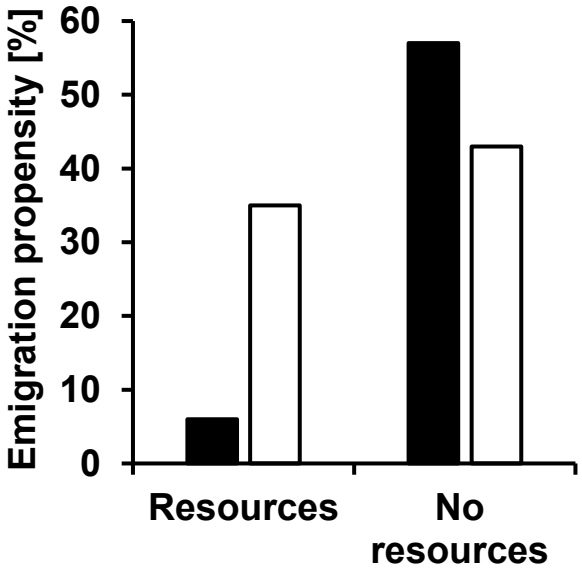

Figure 5 Emigration propensity (%) in relation to the presence or absence of resources in the departure cage in *Lycaena tityrus*. Males: filled bars; females: open bars

This suggests that dispersal in *L. tityrus* is not random but an active process, following an accurate assessment of patch quality. Thus, dispersal in this butterfly is a highly plastic, context-dependent trait triggered largely by habitat quality rather than by individual condition (Saastamoinen *et al.*, 2012; Legrand *et al.*, 2015). Climate change is expected to frequently reduce habitat quality, but this will at the same time likely increase emigration propensity and thereby potentially facilitate range shifts in flying insects (Travis *et al.*, 2013). This should be considered when trying to forecast future species distributions.

2.3 Consequences of larval and adult food stress for flight performance and exploratory behavior

Dispersal sets the potential of individuals to spread, for instance away from local unfavorable conditions, to avoid competition or to more suitable habitats (Matthysen, 2012). Anthropogenic global change, including agricultural intensification (fertilization, pesticide use and high mowing frequencies) and climate change (changes in

temperature and precipitation), poses a substantial challenge to many herbivores due to a reduced availability or quality of feeding resources (Parmesan & Yohe, 2003; Pleasants & Oberhauser, 2013; Lebeau *et al.*, 2016). Resulting food stress is expected to detrimentally affect performance, amongst others in dispersal-related traits as flight is a highly energy demanding process (Candy *et al.*, 1997; Suarez, 2000). Larval food stress may additionally shape the adult's physiological state due to a reallocation of resources among competing body parts and functions such as reproduction, storage, and flight-related traits (McNamara & Houston, 1996; Boggs, 2009). Further, conditions experienced during development may affect the adult phenotype as a predictive adaptive response in order to be better prepared for dealing with detrimental conditions in later life (e.g. Monaghan, 2008). This has already been demonstrated for dispersal ability through alterations in body composition or behavior (Saastamoinen *et al.*, 2010; van den Heuvel *et al.*, 2013).

Therefore, this study investigated the impact of larval and adult food stress on traits related to dispersal ability in the butterfly *Lycaena tityrus*. In the larval food stress treatment, individuals experienced a period of food deprivation of 30 h to mimic a situation in which a larva has to leave a deteriorated host plant and search for a new one. To implement adult food stress, butterflies were exposed to a period of food deprivation for 2 days (i.e. the period from eclosion to testing), during which they were provided with water only. Following the treatments, all butterflies were first subjected to a tunnel test, testing for exploratory behavior, a trait that may reflect behavioral differences in butterflies (Ducatez *et al.*, 2012, 2014). Afterwards, butterflies were subjected to a flight performance test (Ducatez *et al.*, 2012; Legrand *et al.*, 2015). Both tests were conducted at different ambient temperatures. Additionally, several morphological and physiological traits were investigated (see 2.2).

The study revealed that inadequate nutrition during development and in the adult stage diminishes flight performance, despite some reallocation of somatic resources in favor of dispersal-related traits (Fig. 6a). Similar results were found for exploratory behavior (Fig. 6b). These detrimental effects seem to be mainly caused by reductions in body mass and storage reserves, which may have important implications for dispersal in natural environments. While deteriorating habitat conditions are expected to promote dispersal as an adaptive response (Legrand *et al.*, 2015), they may at the same time

impair flight ability and thereby likely dispersal rates. Higher temperatures increased flight performance, demonstrating the strong dependence of flight performance and presumably also dispersal on environmental conditions (Fig. 6a). As shown before (see 2.2), the sexes differed in morphology.

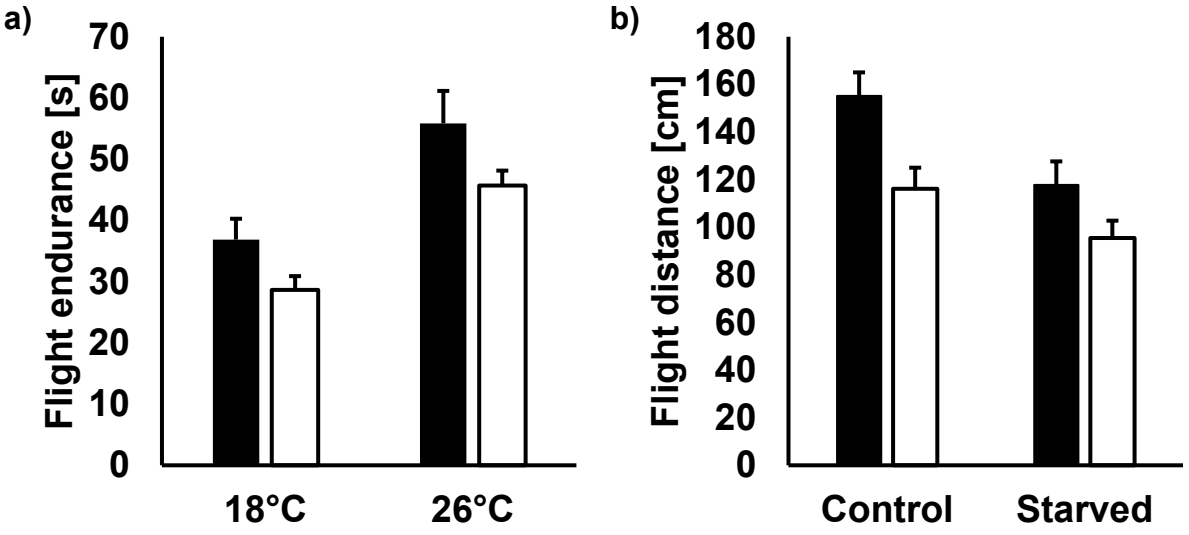

Figure 6 Flight endurance in a vortex (a, means + 1 SE) in *Lycaena tityrus* in relation to adult feeding treatment and test temperature. Food: filled bars; no food: open bars. Flight distance covered in a tunnel (b, means + 1 SE) in *Lycaena tityrus* males and females in relation to larval feeding treatment. Males: filled bars; females: open bars

Exploratory behavior was found to be (moderately) repeatable at the individual level. Furthermore, flight performance and exploratory behavior were positively correlated, probably indicating the existence of a dispersal syndrome (e.g. for *Pieris brassicae* in Ducatez *et al.,* 2012). Thus, the individuals performing repeatedly better than others in different types of flight tests might be the ones more likely to disperse (Delattre *et al.*, 2013).

2.4 Comparison between core and edge populations

Human-induced environmental changes, like rising temperatures or habitat degradation, force species to respond. Next to phenotypic plasticity, which allows species to tolerate novel conditions, and genetic adaptation, dispersal and concomitant

range shift is one possible option (Parmesan, 2006; Berg *et al.*, 2010; Hofmann & Todgham, 2010). Dispersal allows individuals to escape unfavorable conditions, the colonization of new areas, and affects patterns of local adaptation (Doebeli & Dieckmann, 2003; Hickling *et al.*, 2006; Baguette *et al.*, 2013). Evidence suggests that dispersing individuals may differ in a set of phenotypic traits from conspecifics, indicating the existence of a dispersal syndrome (Bonte & Saastamoinen, 2012; Stevens *et al.*, 2013). Assuming that recent populations are founded by the most dispersive individuals (cf. Hill *et al.*, 1998; Hanski *et al.*, 2002, 2004) and dispersal-related traits are heritable (Roff & Fairbairn, 2001), more dispersive genotypes should accumulate in recently established populations (Shine *et al.*, 2011). Besides a good dispersal capacity, the ability to establish populations beyond the former range, once suitable habitats have been reached, is of crucial importance. Different environmental condition at range margins might require local adaptation (Hoffmann *et al.*, 2002; Castañeda *et al.*, 2005). Thus, phenotypic differences between recently established edge and core populations are expected, especially in traits related to dispersal, due to differences in selection pressures and genetic structure (Volis *et al.*, 1998; Purves, 2009; Mägi *et al.*, 2011).

Against this background, the study compared replicated core (Germany) and recently established edge (Estonia) populations of the currently northward expanding butterfly *Lycaena tityrus* (Brunzel *et al.*, 2008; Settele *et al.*, 2008; Klockmann *et al.*, 2016), which has colonized north-eastern Estonia very recently. The aim was to explore variation in dispersal ability and life history traits indicative of local adaptation. Larvae of both countries were reared at control (26°C, 60% relative humidity, L18:D6 photoperiod) and cool (18°C, 60% relative humidity, L18:D6 photoperiod) conditions. One day-old butterflies were first subjected to a behavioral experiment, testing for the individual's exploratory behavior towards a food source in an unknown experimental setup. On the following day butterflies were tested for flight performance (Ducatez *et al.*, 2012; Legrand *et al.*, 2015). Additionally, morphological and physiological parameters were investigated (see above, Berwaerts *et al.*, 2002).

The results show that sexes differed in developmental, morphological, and physiological traits (e.g. Gilchrist, 1990; Berwaerts *et al.*, 2006). In general, males showed traits associated with increased flight ability, and enhanced exploratory

behavior, which might be helpful to quickly establish a territory (Fischer & Fiedler, 2000; Saastamoinen *et al.*, 2012). Despite the very recent colonization, several differences between edge and core populations indicative of local adaptation were found. Edge populations were characterized by shorter development times and smaller size, a higher sensitivity to high temperature and an enhanced exploratory behavior (Fig. 7).

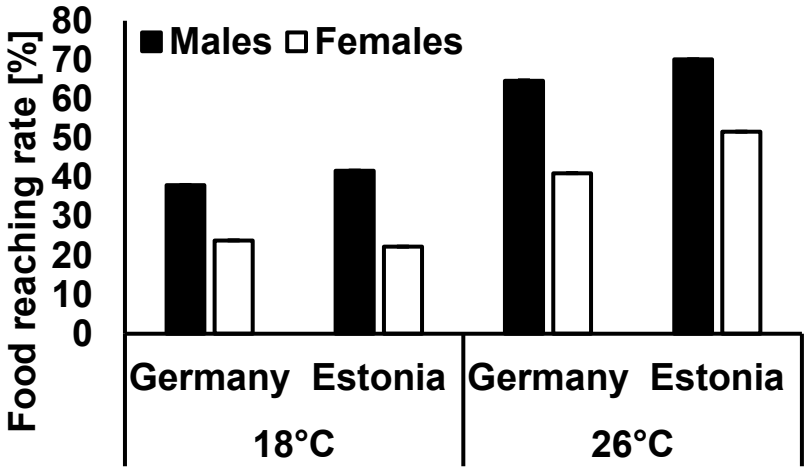

Figure 7 Exploratory behavior (animals finding a food source within 90 minutes in %) in *Lycaena tityrus* males (filled bars) and females (open bars) in relation to origin (country) and developmental temperature.

These differences seem to partly reflect adaptations to the cooler Estonian climate and the concomitantly short vegetation period (or a loss of adaptation to warmer conditions). Enhanced exploratory behavior can be advantageous in all steps of the dispersal process and may have facilitated the current range expansion. It is currently unclear whether the above differences reflect clinal variation or an accumulation of cold-adapted and / or more dispersive genotypes in north-eastern edge populations. A contribution of parental effects can also not be excluded (Wolf *et al.*, 1998; Mattila & Hanski, 2014). In summary, this study provides knowledge which might be useful for a better understanding of species responses to environmental change, in particular insights into the factors associated with a current range expansion.

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PUBLICATION LIST

4 Publication list

- **Reim, E.**, Arnstedt, I., Barwisch, I., Baumgarten, M., Bock, S., Eberspach, J., Ellerbrok, J., Gebremeskel, M., Küpper, S., Guth, L., Lassen, A., Letro, L., Meth, R., Möller, M., Närmann, F., Neunaber, I., Seliger, A., v. Stein, W., Vallinga, C., Vögele, P., Wagner, M., Fischer, K. (2018) Movement patterns differ between sexes and depend on weather conditions in the butterfly *Lycaena tityrus*. Journal of Insect Behavior, 31(3), 309-320.

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All authors conceived the ideas and designed methodology; IA, IB, MB, SB, JE, JE, MG, SK, LG, AL, LL, RM, MM, FN, IN, AS, WS, CV, PV and MW collected the data; **ER** analyzed the data; IA, IB, MB, SB, JE, JE, MG, SK, LG, AL, LL, RM, MM, FN, IN, AS, WS, CV, PV and MW wrote the first draft of the manuscript. **ER** and KF revised and edited the manuscript.

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Movement Patterns Differ between Sexes and Depend on Weather Conditions in the Butterfly *Lycaena tityrus*

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Abstract Habitat loss and anthropogenic climate change are important threats to biodiversity conservation. Owing to the concomitantly deteriorating habitat quality, individuals are often forced to disperse to new habitats, rendering dispersal an ecologically important process. However, dispersal ability may differ within and among populations, and is further dependent on environmental conditions. We therefore studied sexual differences in and environmental effects on movement patterns in the sooty copper butterfly *Lycaena tityrus*. As predicted, males were more active and covered longer distances than females, presumably owing to mate location and territorial disputes. Males alighted more often on flowers than females, probably to fuel their high flight activity, while females showed a high affinity to host-plants for egg-laying. Our findings provide a striking example of sex-related differences in animal behavior, as revealed by the use of customary smartphones, which apparently can comprise suitable means to reveal biologically significant behavioural patterns. More problematic than the technical device used seems to be the challenge of following individual butterflies for long enough in the field, such that any extrapolations to dispersal seem difficult.

Keywords Animal behavior · dispersal · environmental context · flight activity · mate location · sexual differences

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Introduction

Human impact, driving habitat loss and fragmentation as well as climate change, is all pervasive on Earth (e.g. Forester and Machlist 1996; Sanderson et al. 2002). Owing to the concomitantly deteriorating habitat quality, individuals are often forced to disperse to new habitats (e.g. Hanski 1998), although the likelihood to reach these is often low due to reduced habitat availability and fragmentation (Fernández-Chacón et al. 2014). Dispersal is therefore important for dealing with environmental variation (Cote and Clobert 2007; Travis et al. 2013), as it may allow individuals to track their shifting climate niche (Warren et al. 2001; Hickling et al. 2006) and to maintain metapopulation connectivity (Hanski and Gilpin 1997; Baguette et al. 2013).

Despite its concomitant ecological importance, the motivation as well as the ability to disperse may differ strongly among and within populations (Van Dyck and Baguette 2005; Baguette and Van Dyck 2007; Ducatez et al. 2014; Bestion et al. 2015). Factors that may affect dispersal ability include morphology, physiology, and overall condition (Bowler and Benton 2005; Bonte et al. 2012; Therry et al. 2014). Moreover, sexes may differ in dispersal behavior. In insects, females are often the more dispersive sex (e.g. Fischer et al. 1999) as (1) single-mated females are, unlike males, able to found a new population (Hill et al. 1999; Hanski et al. 2002, 2004), (2) females may need to escape from male harassment (Hovestadt and Nieminen 2009; Trochet et al. 2013) or as (3) they pursue a risk spreading strategy by distributing their eggs over a wider area (Hopper 1999). In addition to such intrinsic factors, dispersal is also affected by external factors such as prevailing weather conditions (Legrand et al. 2015). In general, dispersal increases with rising temperature and solar radiation but decreases with increasing cloud cover, rainfall, and wind speed (Kuussaari et al. 2016).

Against this background, we here investigate (within-patch) movement patterns in the temperate-zone butterfly *Lycaena tityrus*. Focussing on movement patterns rather than dispersal per se was due to the fact that dispersal is very difficult if not impossible to observe directly in flying insects (Riley and Smith 2002; Cant et al. 2005). We thus argue that knowledge on movement patterns is an important first step for a better understanding of dispersal, as movement may eventually result in dispersal. For instance, flight track length has been shown to be associated with flight metabolic rate and PGI genotype, parameters that are in turn known to be related to dispersal (Niitepõld et al. 2009). Butterflies are suitable models to study movement for several reasons. First, they exhibit large variation in dispersal ability and their natural history is well known (Stevens et al. 2010). Second, they are sensitive indicators of habitat change and therefore ecosystem quality (Murphy et al. 1990; van Swaay 1990; Erhardt and Thomas 1991). Third, as pollinators they play an important role in ecosystems by providing pollination services, the lack of which may cause a loss of plant diversity (Potts et al. 2010). Fourth, as herbivores they may be particularly threatened by climate change, due to effects on the quality and phenology of host-plants as well as on the butterflies themselves (Roy and Sparks 2000).

Specifically, we explore within-patch mobility to test the hypotheses that (1) movement patterns differ among the sexes, and that (2) movement is strongly affected by prevailing weather conditions. As *L. tityrus* males are territorial (Ebert and Rennwald 1991; Fischer and Fiedler 2000a), we predict that males are more active than females (due to mate location, inspection flights, territorial disputes), while females are

expected to move further away from the point of first encounter than males due to straighter flight trajectories (see above). We further predict that activity is positively related to beneficial (warm, sunny) weather conditions. Addressing such questions may have important implications for dispersal, though note that we are working here on a spatially very restricted scale. To this end, we individually tracked butterflies of both sexes within an extended habitat patch and recorded weather conditions in parallel. As we used customary smartphones in combination with a tracking application, we additionally investigate whether such devices are suitable for investigating insect behaviour.

Material and Methods

Study Organism

The sooty copper *L. tityrus* is a widespread butterfly of the temperate zone, ranging from Western Europe to Central Asia (Ebert and Rennwald 1991). Central European low-altitude populations, including the one investigated here, are typically bivoltine with two distinct generations per year. *L. tityrus* inhabits different types of unimproved grass- and wetlands. The most important larval host plant is *Rumex acetosa*, but several congeneric plant species such as *R. acetosella* and *R. scutatus* are utilised as well (Ebert and Rennwald 1991; Tolman and Lewington 1998). Adults feed on a variety of composite (Compositae) and other nectar plants (Ebert and Rennwald 1991; Karl and Fischer 2009). For this study, individuals from a low-altitude population near the city of Greifswald (north-eastern Germany) were examined.

Study Area

The study was conducted on an extended set-aside field near Greifswald (54°03′02”N, 13°26′26″E), harboring a large population of *L. tityrus*. Hence, the location is close to the Baltic Sea and thus the northern distribution limit of *L. tityrus* (GfS 2017). This part of Germany is characterized by a rather continental climate with an annual mean temperature of 8.0 °C and a precipitation of 566 mm per year (Müller 2006). The study area (ca. 20 ha) is situated 30–40 m above sea level, rather flat, and shows a relatively homogenous vegetation structure without shrubs or trees. The vegetation consists mainly of species indicative of rather nutrient-poor grassland. Host plants (*R. acetosa*, *R. acetosella*) occur in high density throughout the area. The lack of barriers and other disturbing structure renders this study area suitable for tracking individual butterflies.

Field Methods and Data Analyses

We investigated the movement patterns of *L. tityrus* by tracking the flight paths of individual butterflies with a hand-held GPS device. As GPS device we used Android smartphones and the application “Geo Tracker – GPS tracker”. The following settings were used: accuracy 10 to 20 m, frequency of locating 1 per sec, minimal distance between two points 1 m, maximum distance between two points 500 m. To test for the

accuracy of the GPS application and the smartphones used, tracks of a known distance were compared to measured GPS tracks (Online Resource Fig. 1), revealing a correlation coefficient of 0.995. Furthermore, repeatedly scoring the same position using Geo Tracker without moving revealed identical coordinates. Field work was conducted between mid-May and early June 2017, i.e. within the spring flight period of *L. tityrus*. Data were collected by 12 teams consisting of two persons each, which were consistent over the study period. One person concentrated on observing and tracking the butterfly while the other noted butterfly behavior, time periods, etc. (see further below).

To start a track, an individual of *L. tityrus* was located and its sex determined. Thereafter, the observation was started (to ease sex determination and avoid chasing butterflies, all tracks were started when butterflies were sitting). The observers kept a minimum distance of 2–3 m to the butterfly to minimize possible influences on the animal's behavior. Nevertheless, the actual flight path was tracked as closely as possible by avoiding any shortcuts. Tracking of a butterfly ended when the observer lost sight of the individual or after a maximum of 15 min (i.e. tracking was terminated in case an individual was still available after 15 min). Butterflies showing no flight activity within 15 min were omitted from further analyses.

The following parameters were recorded during tracking: date, sex, cloud cover on a scale between 1 and 8 (in n/8; thus 1/8 equals a cloud cover of one eighth of the sky and 7/8 cloudless), start and end time of tracking, the number of stops, the substrate at the place of alighting (either bare ground, host plant, nectar flower, or other vegetation). Thus, stops separated different flight steps, while crawling within the vegetation was not considered as movement. In total 196 tracks were taken, 106 from males and 90 from females, with each of the 12 observer teams contributing ≥10 tracks. The tracking application was used to extract the following data: time in motion, total track length, average movement speed, average movement speed in motion, and maximum movement speed. To assess whether a high activity may facilitate dispersal, we additionally measured the linear distance between the starting and end point of each track in Google Earth by exporting the tracks to a computer device. Step length was calculated as total track length divided by the number of stops. Data on temperature, wind speed, relative humidity, and precipitation during each individual track were obtained from the Deutscher Wetterdienst (DWD 2017) and are based on hourly means of the weather station closest to the study area (station Greifswald, distance 5.5 km, coordinates: 54°05′39”N, 13°24′20″E).

Statistical Analyses

Considering the high density of *L. tityrus* and the large size of the study area, we deem it unlikely that a single individual was observed more than once, although we did not mark the individuals. Therefore, we consider our observations as independent samples representative of the butterfly behavior in this population. To analyze movement-related traits we used general linear mixed models with sex as fixed effect, observer team as random effect, and the covariates tracking time, cloud cover and temperature. Due to correlations with temperature, wind speed and relative humidity were excluded from further analyses. Additionally, precipitation was not considered as it showed essentially no variation, because field work was not carried out under rainy conditions (Online Resource Table 1 and Fig. 2). All statistical tests were performed with Statistica 12.0 (Tulsa, StatSoft, OK). All means are given ±1 SE.

Results

Significant sex differences were found in track length, linear distance between start and end point, time in motion, average speed, average movement speed, and the number of stops on flowers and on host-plants, but not in step length, maximum speed, and the number of stops in total, on other vegetation or on bare ground (Table 1). On average, males compared with females exhibited longer track lengths (Fig. 1a) and distances between start and end point (22.3 ± 2.5 m vs. 14.5 ± 1.6 m), higher average speeds over the total observation period (0.78 ± 0.08 km/h vs. 0.22 ± 0.03 km/h) and also in motion (Fig. 1b), spent more time flying (Fig. 1c), and stopped more often on flowers (Fig. 1d) but less often on host-plants (Fig. 1e).

Regarding effects of environmental factors, only linear distance between start and end point, step length, and the number of stops on host-plants were significantly associated with cloud cover, indicating that increasing cloud cover increased distances (beta 0.17 ± 0.09, Online Resource Fig. 3) and step lengths (beta 0.18 ± 0.09) but decreased the number of stops on host-plants (beta −0.17 ± 0.08). Temperature exclusively affected the number of stops on bare ground, which increased with decreasing temperature (beta −0.36 ± 0.11). Track length (beta 0.17 ± 0.08), the total number of stops (beta 0.44 ± 0.07) and the number of stops on flowers (beta 0.23 ± 0.08), host-plants (beta 0.22 ± 0.08) and other vegetation (beta 0.32 ± 0.08) were all significantly positively related, whereas step length (beta −0.19 ± 0.09) and average speed (beta −0.36 ± 0.07) were negatively related to tracking time, while no significant association was found with any of the other dependent variables. Significant effects of observer team were found in all dependent variables except from step length and the number of stops on bare ground.

Discussion

Our study revealed, as predicted, sex-specific differences in behavioral patterns of *L. tityrus*. Males spent more time flying and showed longer track lengths than females, in accordance with our expectation. We assume that the longer track lengths result from the males' higher overall activity (see also Kingsolver 1983). The males' higher activity is most likely due to spending much of their time locating mates including frequent inspection and patrolling flights as well as territorial disputes with rivalling males (Wickman 1985; Ebert and Rennwald 1991; Fischer et al. 1999). Females, in contrast, seem to generally spend less time flying and allocate more of their time to oviposition (Kingsolver 1983; Fischer et al. 1999). However, the fact that males also covered longer linear distances between the start and end points of observations is contrary to our expectation. For various reasons we expected females to cover longer distances owing to straighter flight trajectories, while males were supposed to stay within or at least close to their territories. Furthermore, the current data challenge earlier ones obtained from mark-recapture studies in related species, where males were found to be more stationary than females (Fischer et al. 1999; Fischer and Fiedler 2000b). We believe that our current results are biased due to the rather short observation periods (max. 15 min) compared to the mark-recapture studies in which butterflies were observed for several days. Thus, within this rather short period of time, the males'

Table 1 General linear mixed models for the effects of sex (fixed), observer team (random), and the covariates tracking time, cloud cover and temperature on track length, linear distance between start and end point of track, step length, time in motion, maximum speed, average speed, average movement speed, total number of stops, the number of stops on flowers, the number of stops on host-plants (*Rumex* spec.), the number of stops on other vegetation, and the number of stops on bare ground in the butterfly *Lycaena tityrus*. Significant *p*-values are given in bold

Track length	DF	MS	F	p
Sex	1	55324	29.9	**< 0.0001**
Observer team	11	7336	4.0	**< 0.0001**
Track time	1	9653	5.2	**0.0236**
Cloud Cover	1	1943	1.5	0.3071
Temperature	1	205	0.1	0.7397
Error	179	1852		
Linear distance	DF	MS	F	p
Sex	1	2443	5.8	**0.0173**
Observer team	11	929	2.2	**0.0165**
Track time	1	6	< 0.1	0.9077
Cloud cover	1	1677	4.0	**0.0480**
Temperature	1	549	1.3	0.2560
Error	177	423		
Step length	DF	MS	F	p
Sex	1	466	1.0	0.3170
Observer team	11	762	1.6	0.0890
Track time	1	2235	4.8	**0.0292**
Cloud cover	1	1852	4.0	**0.0469**
Temperature	1	53	0.1	0.7364
Error	179	462		
Time in motion	DF	MS	F	p
Sex	1	145652	26.3	**< 0.0001**
Observer team	11	18491	3.3	**0.0003**
Track time	1	8850	1.6	0.2077
Cloud cover	1	9056	1.6	0.2025
Temperature	1	11899	2.1	0.1444
Error	179	5536		
Maximum speed	DF	MS	F	p
Sex	1	20.8	2.6	0.1112
Observer team	11	27.8	3.4	**0.0002**
Track time	1	23.1	2.8	0.0936
Cloud cover	1	10.4	1.3	0.2595
Temperature	1	0.1	< 0.1	0.9255
Error	179	8.1		
Average speed	DF	MS	F	p
Sex	1	4.5	14.1	**0.0002**

Table 1 (continued)

Observer team	11	1.5	4.7	**< 0.0001**
Track time	1	8.0	4.7	**< 0.0001**
Cloud cover	1	0.3	0.9	0.3342
Temperature	1	< 0.1	< 0.1	0.8840
Error	179	0.3		
Average movement speed	DF	MS	F	p
Sex	1	39.6	6.5	**0.0115**
Observer team	11	25.2	4.2	**0.0002**
Track time	1	9.6	1.6	0.2104
Cloud cover	1	15.4	2.5	0.1129
Temperature	1	7.5	1.2	0.2687
Error	179	6.1		
Stops number	DF	MS	F	p
Sex	1	13.8	0.7	0.3985
Observer team	11	64.1	3.3	**0.0004**
Track time	1	0.6	34.6	**< 0.0001**
Cloud cover	1	41.9	2.2	0.1423
Temperature	1	32.6	1.7	0.1954
Error	179	19.3		
Flower stops	DF	MS	F	p
Sex	1	70.5	8.7	**0.0035**
Observer team	11	15.3	1.9	**0.0420**
Track time	1	59.7	7.4	**0.0071**
Cloud cover	1	0.4	< 0.1	0.8276
Temperature	1	12.6	1.6	0.2124
Error	179	423.0		
Rumex stops	DF	MS	F	p
Sex	1	62.4	17.6	**< 0.0001**
Observer team	11	8.2	2.3	**0.0115**
Track time	1	18.8	8.1	**0.0049**
Cloud cover	1	17.0	4.8	**0.0300**
Temperature	1	9.5	2.7	0.1037
Error	179	3.5		
Vegetation stops	DF	MS	F	p
Sex	1	9.0	0.9	0.3382
Observer team	11	36.4	3.7	**< 0.0001**
Track time	1	157.1	16.0	**< 0.0001**

Table 1 (continued)

Cloud cover	1	3.1	0.3	0.5749
Temperature	1	2.6	0.3	0.6040
Error	179	9.8		
Ground stops	DF	MS	F	p
Sex	1	< 0.1	< 0.1	0.8934
Observer team	11	0.8	1.7	0.0744
Track time	1	< 0.1	< 0.1	0.8314
Cloud cover	1	0.6	1.3	0.2514
Temperature	1	5.1	11.0	**0.0011**
Error	179	0.5		

higher activity may have caused longer linear distances. Note in this context the short absolute distances covered within the observation period. Additionally, males may have abandoned territoriality. Note that territorial behavior in *Lycaena* butterflies depends on context, and that males may switch to patrolling behavior during spells of beneficial weather conditions and at high densities of conspecifics (Fischer and Fiedler 2001), which was the case during the study period.

The finding that males achieved higher speeds than females has probably morphological and physiological reasons. Male (*Lycaena*) butterflies have a lower body mass and concomitantly wing loading, but a higher thorax-abdomen ratio, wing aspect ratio, and relative fat content compared with females (Karl et al. 2008; Saastamoinen et al. 2012). These traits are typically associated with increased flight ability and maneuverability (van Dyck et al. 1998; van Dyck and Wiklund 2002; Berwaerts et al. 2008),

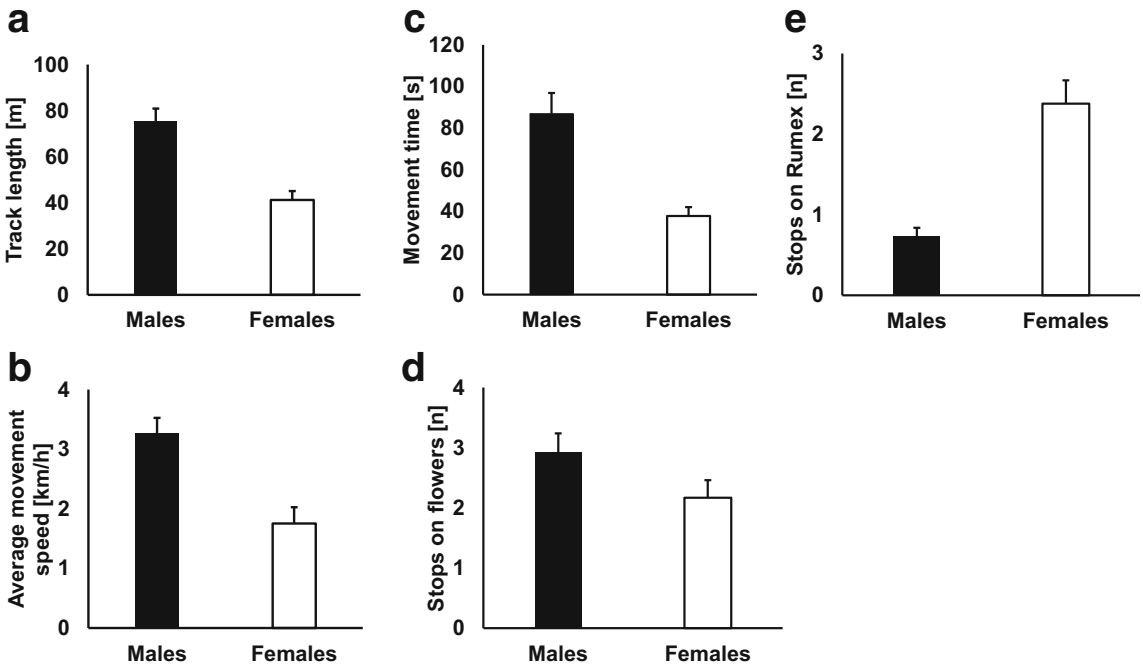

Fig. 1 Sexual differences in *Lycaena tityrus* in track length (**a**), average movement speed (**b**), time in motion (**c**), and the number of stops on flowers (**d**) and *Rumex* host-plants (**e**)

likely of crucial importance for males to succeed in territorial contests and competition for mates. The fact that males alighted more often on flowers than females is presumably caused by the need for nectar to fuel their high flight activity, while the higher affinity of females to host-plants is certainly related to the search for oviposition plants (Fischer et al. 1999).

In contrast to the prevalent sexual differences in movement, effects of environmental factors were less pronounced. While cloud cover significantly affected three variables, temperature affected only one. The negative impact of cloud cover on the number of stops on host plants potentially reflects the high dependence of *L. tityrus* activity on solar radiation, being an extremely heliophilous butterfly (Wickman 2009). Thus, a lack of solar radiation may result in a reduced (egg-laying) activity. Typically, females try to spread their eggs over a wide range as a risk-spreading strategy (Hopper 1999). Contrary to our assumptions, the linear distance covered and step length were positively related to cloud cover, for which we have no straightforward explanation. Perhaps air temperature was still high enough to sustain flight activity. The more frequent stops on bare ground at cooler temperatures are probably related to thermoregulatory needs, thus alighting on relatively warm spots compared with the surrounding vegetation (Thomas and Lewington 2010; Pradel and Fischer 2011). Taken together, these findings lend at least some support to the notion that detrimental weather conditions may negatively affect flight activity and therefore dispersal in insects, as has been also found in other studies (Meyer and Sisk 2001; Dennis and Sparks 2006; Cormont et al. 2011; Kuussaari et al. 2016). Ultimately, this dependence rests on the high thorax temperature needed for flight activity (Pollard and Yates 1994), which cannot be maintained without solar radiation or at cool temperatures.

We additionally investigated the effects of tracking time and observer team. The positive relations between tracking time and several variables were expected, and simply reflect that increasing observation time increases track length, the number of stops, etc. The negative relation between tracking time and step length as well as average speed might indicate that it was easier for the observers to follow slow flying butterflies with short step lengths. Effects of observer team were also widespread, though our statistical approach effectively controlled for both sources of confounding effects.

Conclusions

Our results show that customary smartphones can be suitable to reveal biologically significant and well interpretable behavioral patterns in insects. In particularly behavioral differences between the sexes could be readily determined. Similarly, earlier studies investigating butterfly flight using harmonic radar (Cant et al. 2005) but also GPS receivers (e.g. Breed and Severns 2015; Fernández et al. 2016) revealed solid data on butterfly flight behavior. More problematic than the technical device used seems to be the rather short observation period of 15 min at max. The principal problem here is that small flying insects are notoriously difficult to follow in the field (cf. Riley and Smith 2002; Cant et al. 2005). Accordingly, many tracks were even substantially shorter than 15 min. In that respect it is actually quite surprising that our study revealed robust data. We thus

conclude that our approach was well appropriate to reveal sexual differences in and impacts of ambient weather on butterfly behaviour. However, tracking butterflies with handheld devices does not seem to be appropriate to estimate rates of dispersal and dispersal distances, such that any conclusions regarding insect dispersal need to be drawn with the greatest care.

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Electronic supplementary material

Journal of Insect Behavior

Movement patterns differ between sexes and depend on weather conditions in the butterfly *Lycaena tityrus*

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Table 1 Range of tested weather variables (hourly means) during the observation period

	Observed minimum value	Observed maximum value
Temperature [°C]	14.4	28.5
Wind speed [m/s]	1.8	9.5
Relative humidity [%]	38	91
Precipitation [mm]	0	1.5

Fig. 1 Relationship between the exact distance moved and the distance tracked with the application “Geo Tracker – GPS tracker” for 30 tracks with a length of 2.5 to 30 meters (a). The distance measured and the distance tracked were positively correlated (r= 0.9945). Tracks were measured at intervals of 2.5 m using a marked square of 100 m^2 (b), see (b) for an example, a track of 15 m (marked in red)

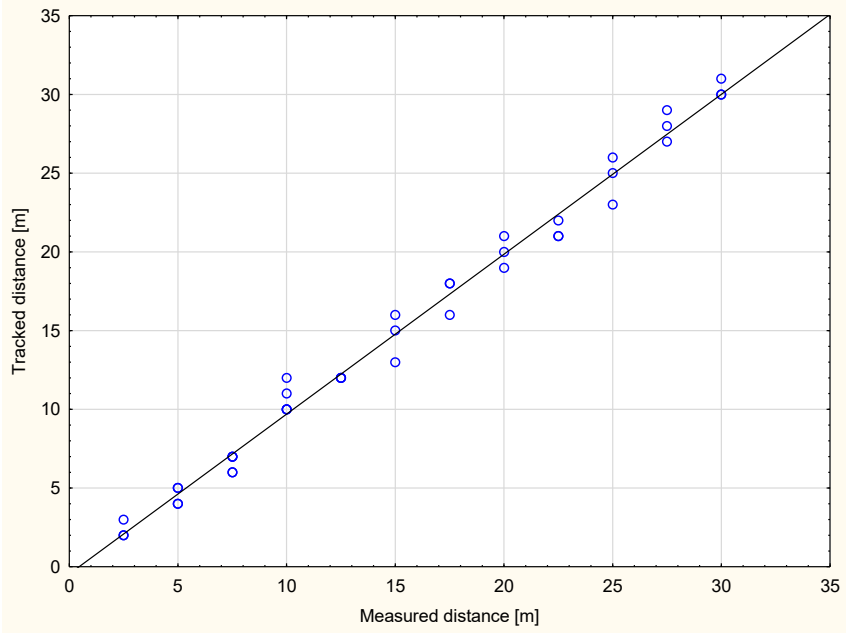

Fig. 1a

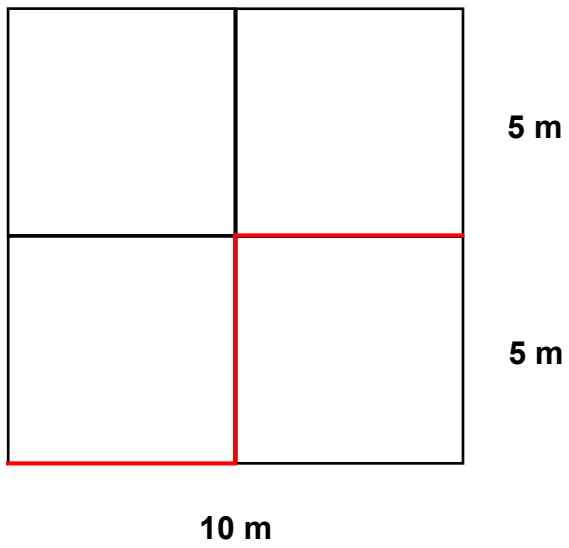

Fig. 1b

Fig. 2 Relationships between the weather variables (a) temperature and wind, (b) temperature and relative humidity, (c) temperature and precipitation, (d) wind and relative humidity, (e) wind and precipitation, (f) relative humidity and precipitation, (g) cloud cover and temperature, (h) cloud cover and wind, (i) cloud cover and relative humidity, and (j) cloud cover and precipitation

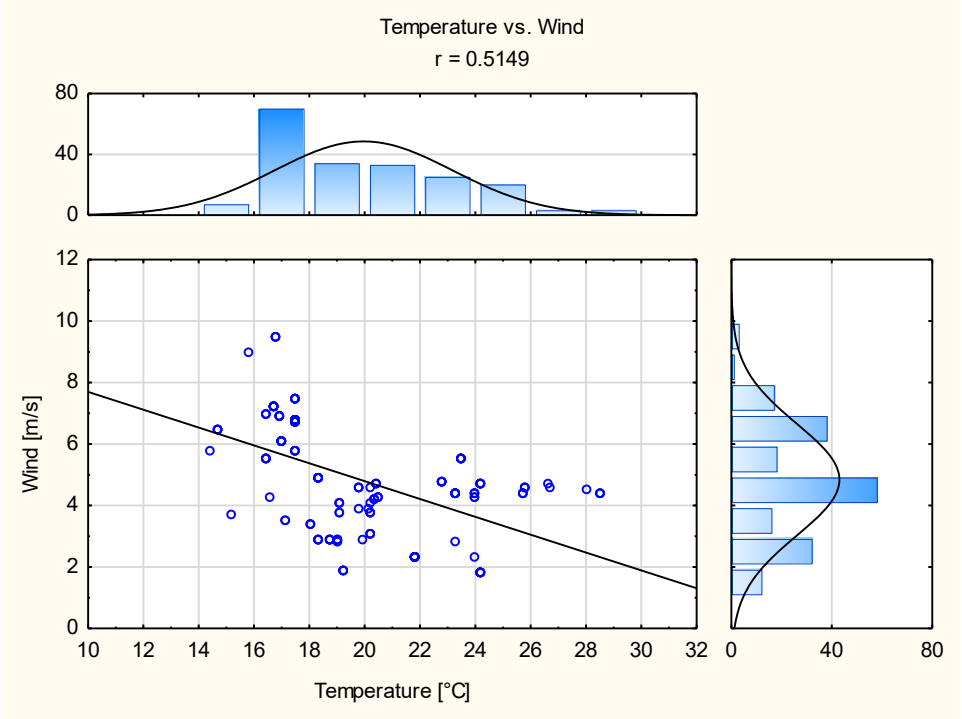

Fig. 2a

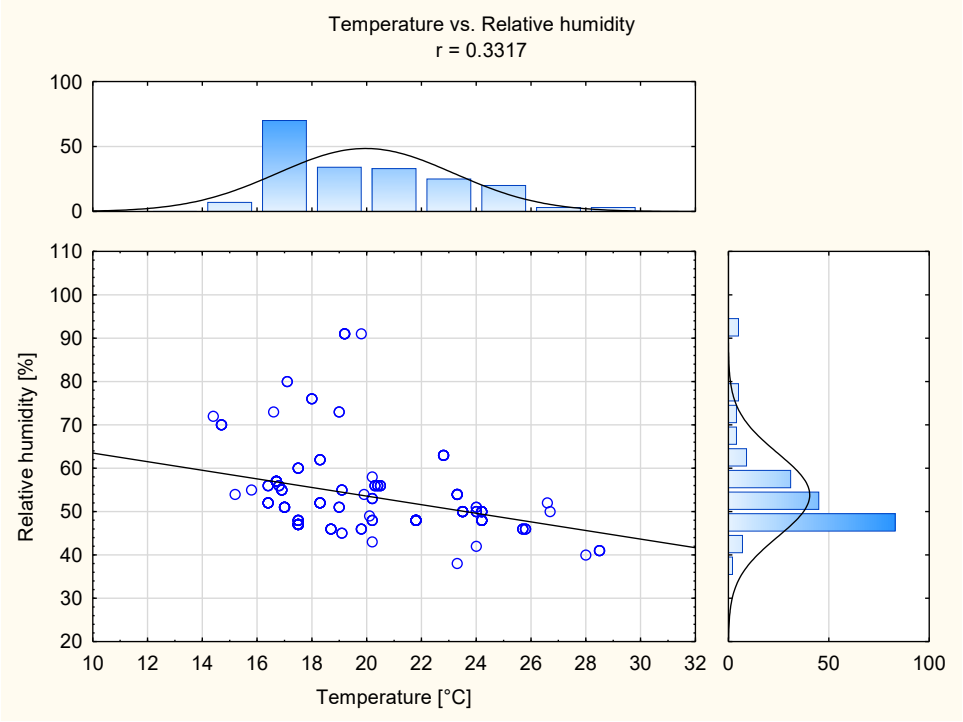
Temperature vs. Relative humidity
r = 0.3317
Relative humidity [%]
Temperature [°C]

Fig. 2b

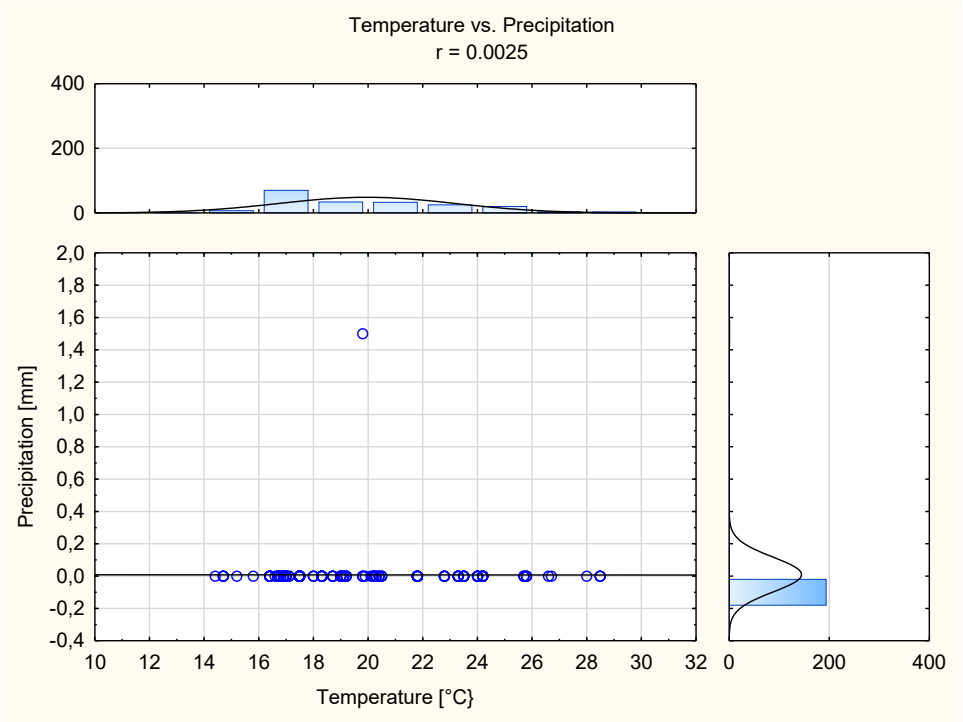
Temperature vs. Precipitation
r = 0.0025
Precipitation [mm]
Temperature [°C}

Fig. 2c

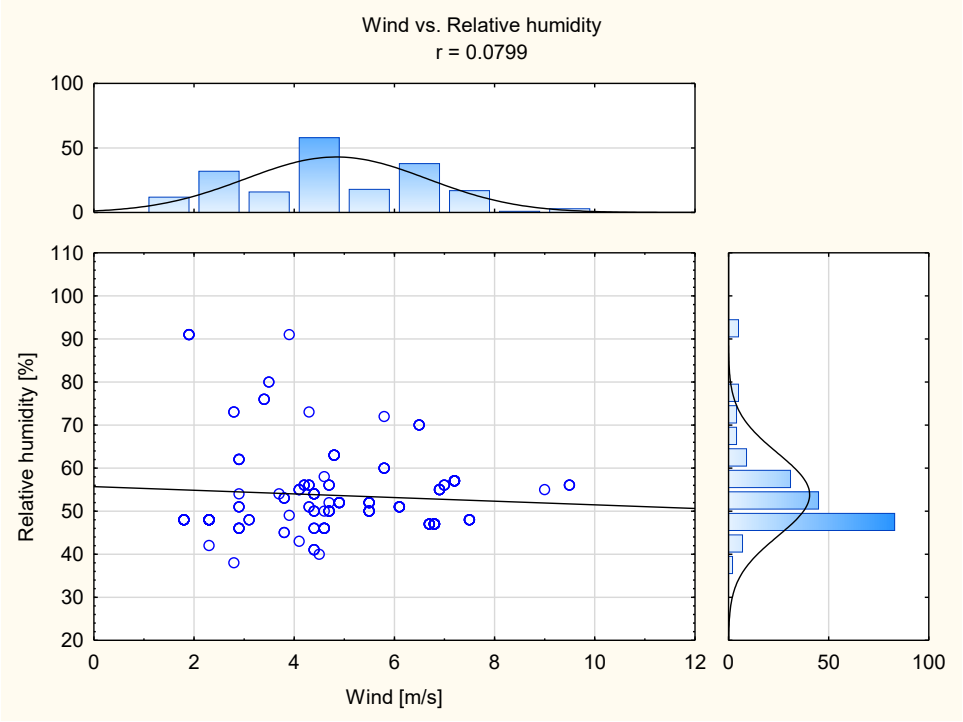
Wind vs. Relative humidity
r = 0.0799
Relative humidity [%]
Wind [m/s]

Fig. 2d

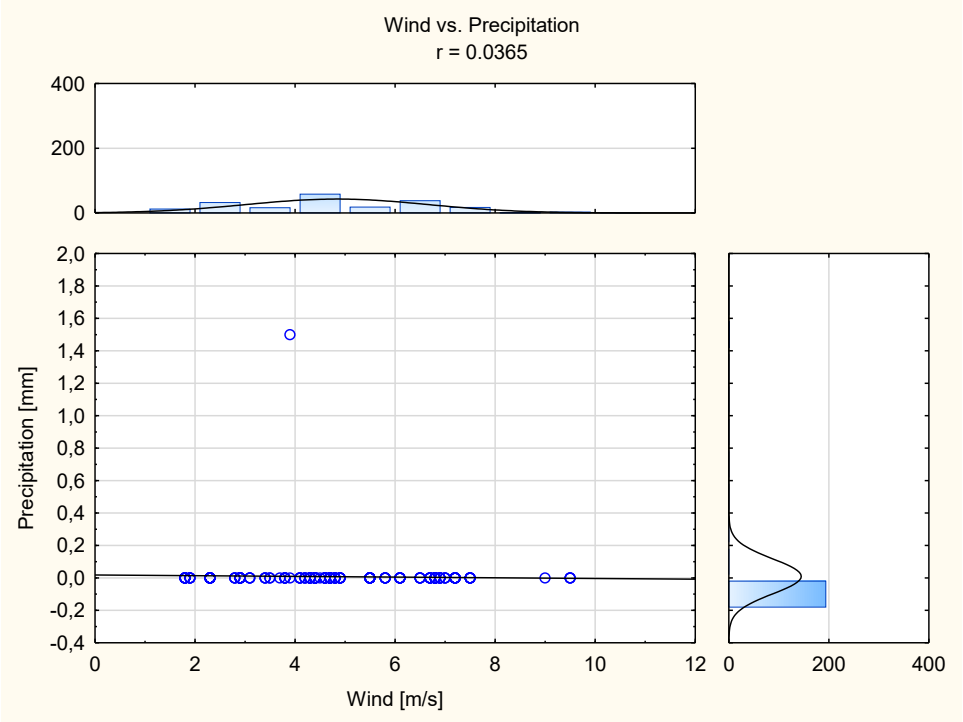
Wind vs. Precipitation
r = 0.0365
Precipitation [mm]
Wind [m/s]

Fig. 2e

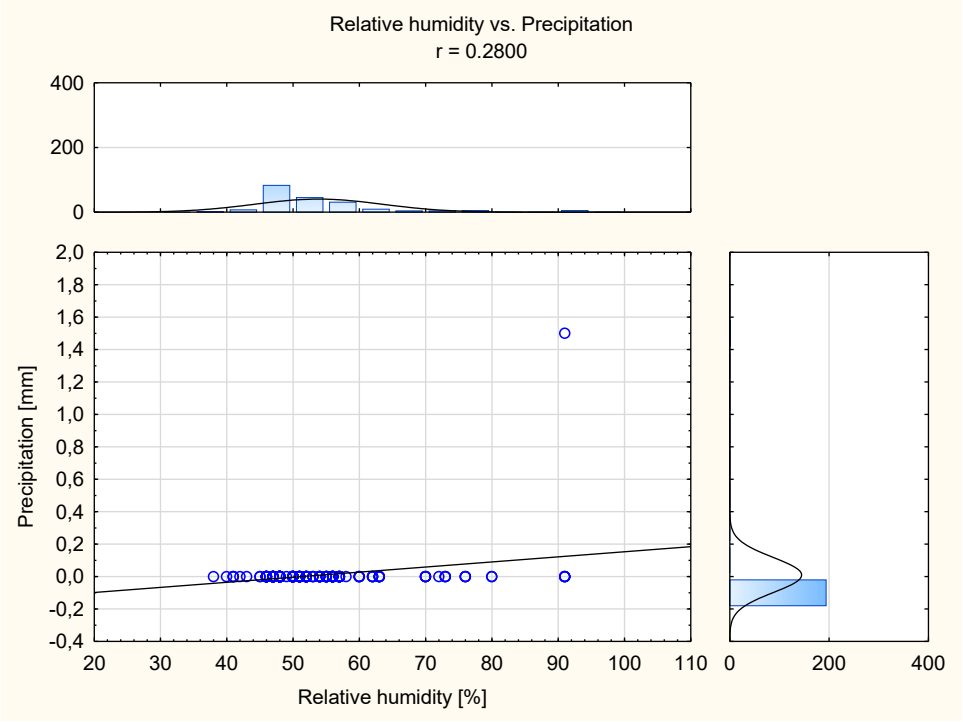
Relative humidity vs. Precipitation
r = 0.2800
400
200
0
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
Precipitation [mm]
20
30
40
50
60
70
80
90
100
110
Relative humidity [%]
0
200
400

Fig. 2f

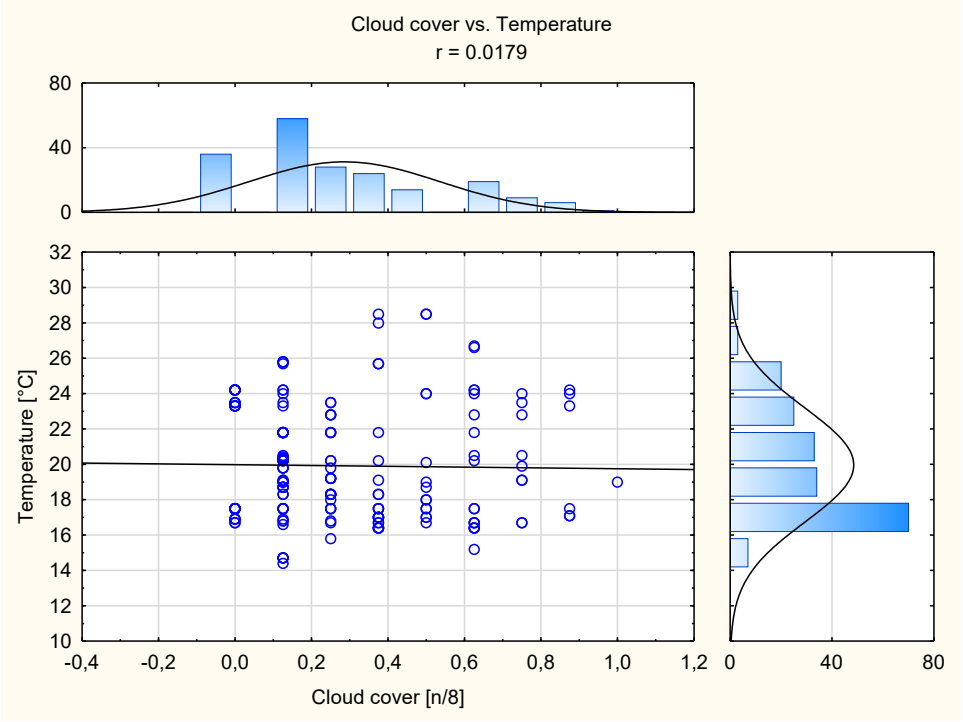
Cloud cover vs. Temperature
r = 0.0179
80
40
0
32
30
28
26
24
22
20
18
16
14
12
10
Temperature [°C]
-0,4
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
Cloud cover [n/8]
0
40
80

Fig. 2g

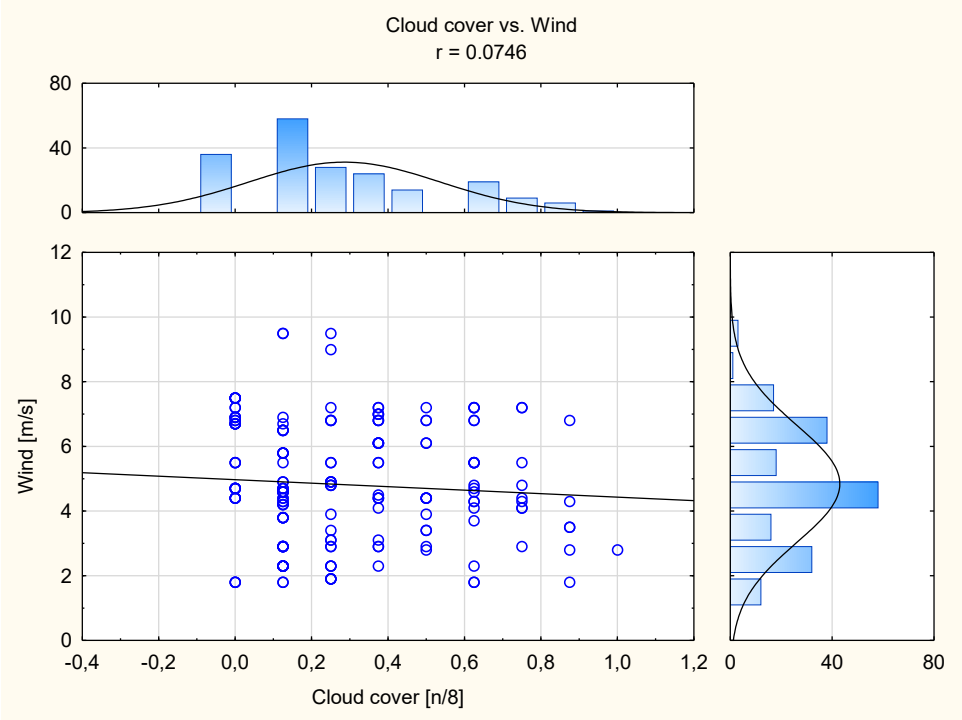
Cloud cover vs. Wind
r = 0.0746
Wind [m/s]
Cloud cover [n/8]

Fig. 2h

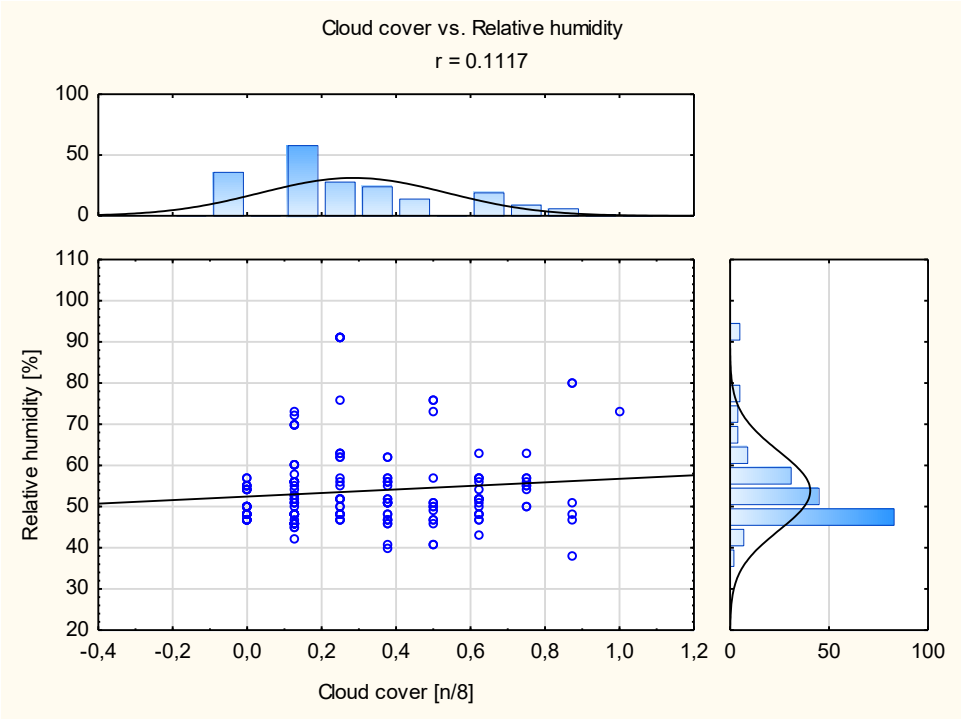
Cloud cover vs. Relative humidity
r = 0.1117
Relative humidity [%]
Cloud cover [n/8]

Fig. 2i

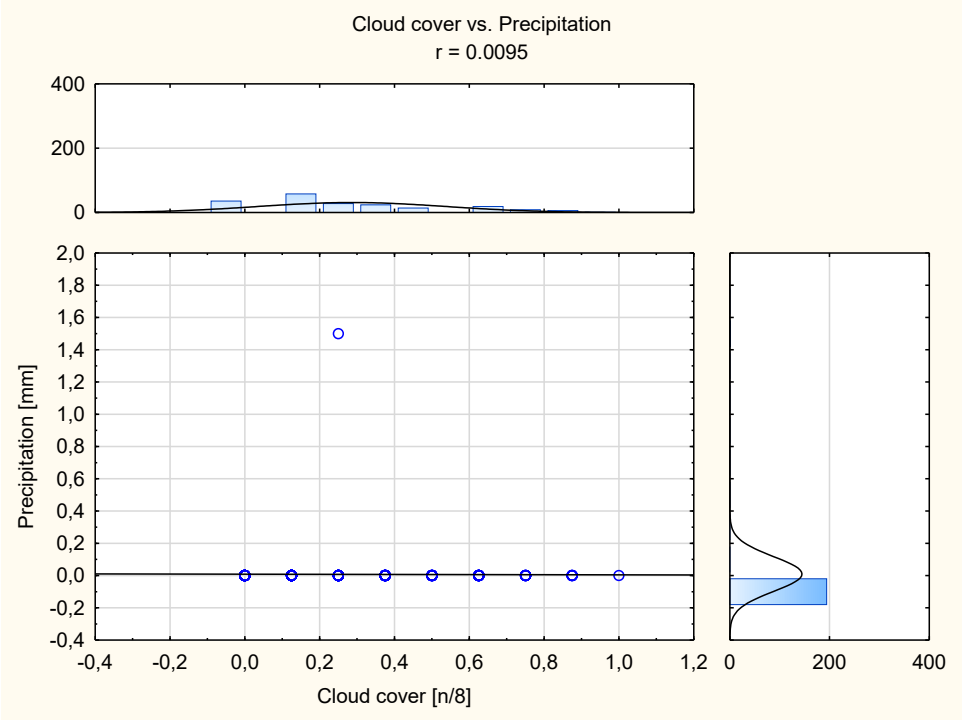
Cloud cover vs. Precipitation
r = 0.0095
400
200
0
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
Precipitation [mm]
-0,4
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
Cloud cover [n/8]
0
200
400

Fig. 2j

Fig. 3 Relationship between the variables (a) linear distance moved and cloud cover and (b) linear distance moved and temperature

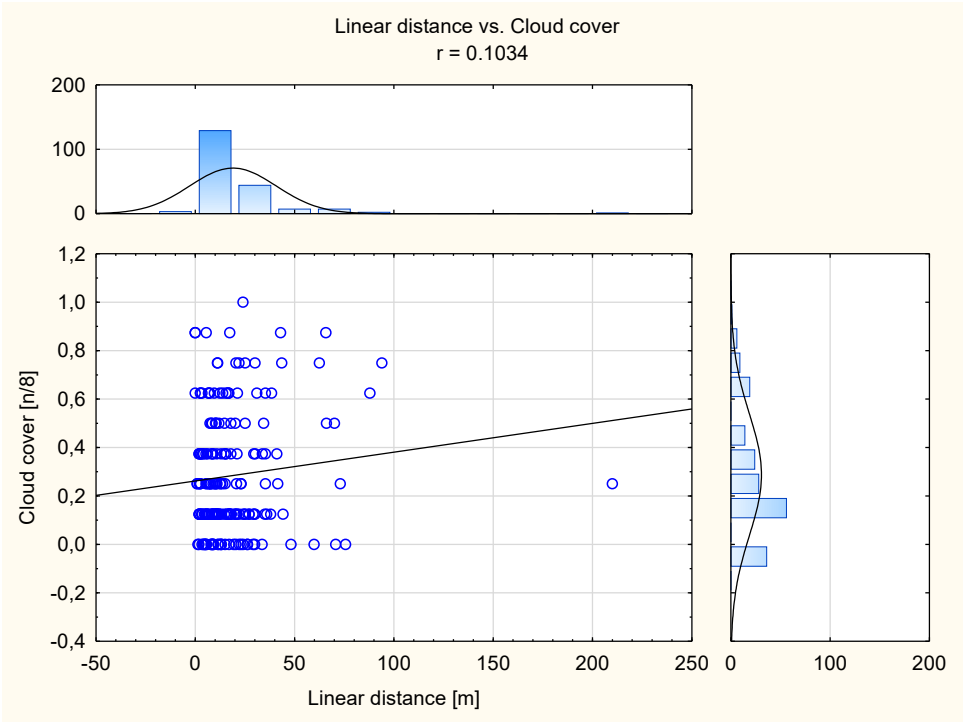

Fig. 3a

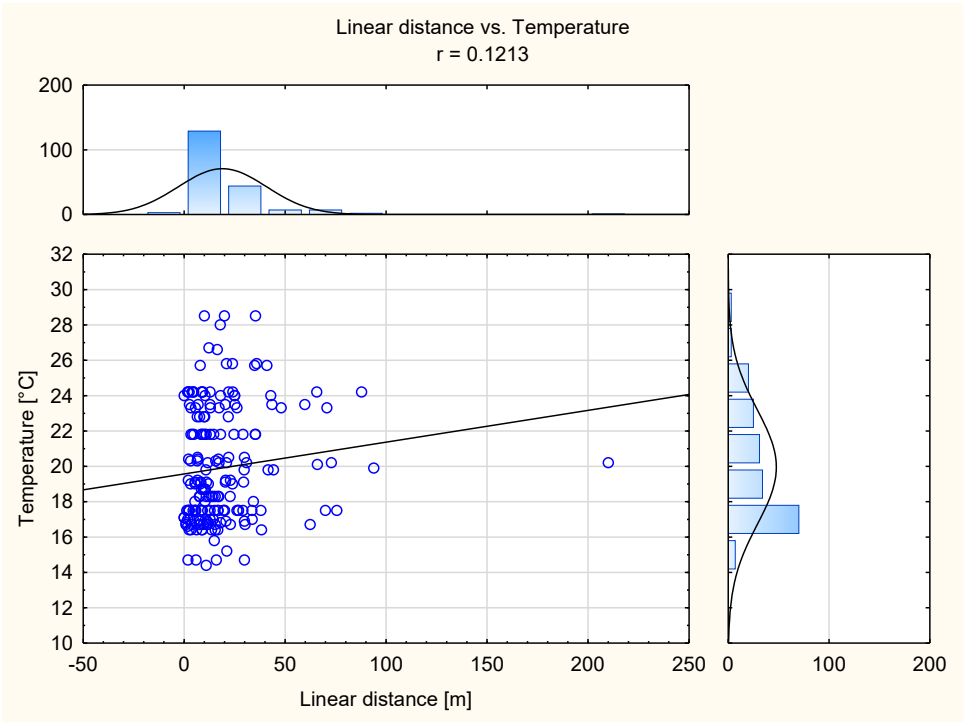

Fig. 3b

4.2 Emigration propensity and flight performance are decoupled in a butterfly

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Running head: Flight behavior in a butterfly

Emigration propensity and flight performance are decoupled in a butterfly

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Abstract. Dispersal, defined as any movements potentially leading to gene flow, is a major process driving a species' capacity to cope with human-induced environmental modifications. However, the dispersal process is multi-causal, which currently hinders predictions regarding a species' resilience to global change. We used a multifaceted approach to disentangle the relative importance of a suite of dispersal-related factors in the butterfly *Lycaena tityrus*, including condition- (morphology, behavior) and context- (environmental) dependent factors. Experiments were conducted at an experimental platform dedicated to study dispersal, the Metatron, to record emigration propensity in two-patch experimental metapopulations under different environmental conditions. The butterflies' individual condition was subsequently assessed in the laboratory. Individual condition did not generally influence emigration propensity. We detected a significant sex-bias in emigration propensity, being in general higher in females than in males, but in a context-dependent manner. The environmental context affected emigration propensity, which was higher when habitat quality was poor. Our results show that emigration is not random in *L. tityrus*, but is rather an active process triggered by sex and habitat quality. Our main finding was that individual condition, and particularly flight ability measured by a performance test, was not related to emigration propensity in experimental metapopulations. Our results may have important implications for forecasting future species distributions, as deteriorating environmental contexts are likely to increase emigration whatever the individual condition is.

Keywords: Climate change; condition; context-dependence; dispersal; flight ability; Lycaena tityrus; metapopulation; Metatron; movement

47 INTRODUCTION

48 Dispersal, often defined as any movements potentially leading to gene flow (Ronce 2007), is a
49 complex process serving different functions and involving a variety of underlying mechanisms
50 (Bowler and Benton 2005, Bonte et al. 2012, Matthysen 2012). The multi-causality of dispersal
51 though has been fully appreciated in recent years only (reviewed by Matthysen 2012),
52 suggesting that complex interactions between several factors rather than a single one influence
53 the dispersal process (Legrand et al. 2015). Currently there is a lack of studies aiming at
54 disentangling the relative importance of multiple factors relevant to dispersal including
55 individual condition (e.g. morphology, behavior) and the environmental context (e.g. habitat
56 quality, weather; but see e.g Legrand et al. 2015, Turlure et al. 2016). Also, growing evidence
57 indicates that dispersal is not a fixed but a highly plastic trait (e.g. Ronce 2007). In invertebrates,
58 for instance, flight behavior is influenced by food availability (e.g. Saastamoinen et al. 2010)
59 and weather conditions (Niitepõld et al. 2009). Consequently, organisms may collect
60 information on their current environment influencing their dispersal decision (Clobert et al.
61 2009). However, only a few studies have focused on such informative cues that are likely to
62 trigger dispersal decisions (but see Cote and Clobert 2007, Baguette et al. 2011).
63
64 The above deficiencies in our current understanding of dispersal are worrying given its crucial
65 ecological and evolutionary importance, ranging from metapopulation dynamics through
66 metacommunity regulation to gene flow and speciation (Clobert et al. 2012). Dispersal is also
67 of utmost importance in driving a species' capacity to respond to human-induced global change
68 like unpredictable climatic variation or habitat loss and fragmentation (Cote and Clobert 2007,
69 Travis et al. 2013). Persistence under such dramatic changes requires a species to either stay
70 and adapt to the new conditions or to leave by moving along the waves of change (Berg et al.
71 2010). In the latter case, dispersal is a key process allowing individuals to track their shifting

climate niche (resulting in species range shifts; Warren et al. 2001, Hickling et al. 2006) or to maintain meta-population connectivity (Baguette et al. 2013).

Against this background, we use a multifaceted approach to uncover the relative role of factors that potentially affect dispersal, including both individual condition (morphology and flight performance) and environmental context. This approach is in line with the conceptual framework for movement ecology developed by Nathan et al. (2008), which integrates the basic components and processes involved in the movement of individual organisms. Consequently, the movement path of an organism results from dynamic interactions between the internal state, motion and navigation capacity, and external factors. As our study organism we used the Sooty Copper butterfly *Lycaena tityrus*, a currently northward expanding species (Brunzel et al. 2008, Settele et al. 2008), rendering the investigation of its movement drivers particularly interesting in the current era of climate change.

We first examined the influence of different environmental factors on the first step of the dispersal process, i.e. the propensity to leave a habitat patch (emigration propensity). Further steps are the transfer phase (movement) and the settlement in a novel habitat (see Bonte et al. 2012 for a thorough description of dispersal as a three step process). We used the Metatron, a unique experimental platform designed to study dispersal in terrestrial organisms in experimental metapopulations (Legrand et al. 2012). Second, we measured flight performance (here: time in flight under stress) to examine connections between emigration propensity under semi-natural conditions and physiological flight performance under laboratory conditions (Ducatez et al. 2012, Legrand et al. 2015). Third, we assessed individual variation in several morphological parameters (e.g. thorax-abdomen ratio, wing loading, fat content) that may be associated with movement ability (Legrand et al. 2016). Note that morphological differences

may have important consequences for flight performance (Arnold 1983, Gilchrist 1990, Berwaerts et al. 2002), and even for dispersal and colonization success in butterflies (Hill et al. 1999, Sekar 2012). How frequently and to what extent such differences directly affect dispersal behavior though is currently not clear. A study of Hanski et al. (2002), for instance, found no effect of morphology on dispersal rates.

We aim to disentangle the relative importance of individual condition versus environmental context to answer the following questions: (1) To what extent is emigration propensity affected by the environmental context potentially indicative of habitat quality? (2) Is emigration propensity in experimental metapopulations related to flight performance, i.e. do individuals with better flight performance emigrate more readily? (3) Which condition-dependent individual factors (e.g. morphology, sex) are related to variation in emigration propensity and flight performance? We predict (1) increased emigration rates if habitat quality is poor, (2) a high correlation between flight performance and emigration propensity, and consequently (3) that flight performance and emigration propensity are tightly linked to morphological traits. Answering these questions might be important for improving predictions on species responses to environmental change.

METHODS

Study organism

Lycaena tityrus (Poda, 1761) is a widespread temperate-zone butterfly ranging from Western Europe to central Asia (Ebert and Rennwald 1991). This species is currently expanding its range towards higher latitudes and altitudes, which is assumed to be largely driven by anthropogenic climate change (Brunzel et al. 2008, Settele et al. 2008). The species is bivoltine with two

discrete generations per year in most parts of its range, although populations with one or three generations per year occur. Overwintering takes place as half-grown larva. The principal larval host-plant is *Rumex acetosa L*. (Polygonaceae), but several congeneric *Rumex* species are utilized as well (Ebert and Rennwald 1991, Tolman and Lewington 1998, Settele et al. 2008). Adults are nectar feeders (Ebert and Rennwald 1991). For the following experiments, a total of 35 freshly eclosed, mated females from a bivoltine German population (vicinity of Greifswald, north-eastern Germany; 54°02N, 13°26E) were caught in June 2015. All females were transferred to a climate chamber at Greifswald University for egg laying.

Animal rearing

Caught females were kept at 26°C, 60% relative humidity, and a L18:D6 photoperiod. For oviposition, females were placed separately in translucent 1 L plastic pots covered with gauze, and were provided with *Rumex acetosa* (oviposition substrate), fresh flowers, water, and a highly concentrated sucrose solution (for adult feeding). Deposited eggs were collected daily and transferred, separated by female, to small plastic boxes and kept under the same conditions used for oviposition. After hatching, larvae were placed in groups of 10-15 individuals in larger, transparent plastic boxes (500 ml), containing moistened filter paper and fresh cuttings of *R. acetosa* in ample supply. Boxes were checked daily and larvae were supplied with fresh food as necessary. Resulting pupae were transferred to a climate cabinet set to 15°C (60% r.h., L18:D6) in order to retard further development. Once most larvae had pupated, pupae were transferred to the 'Station for Theoretical and Experimental Ecology' (SETE) of the CNRS in Ariège, south-western France. There, pupae were randomly divided into three groups and initially also kept at 15°C. Three days before the start of the respective experiment (see further below), pupae were transferred to warmer conditions (26-28°C) to facilitate quick and synchronized adult eclosion. After adult eclosion, butterflies were individually marked by

writing a number on their left hindwing using a water-proof felt-tip pen. Sexes were kept separately until the start of experiments. Butterflies were collected over up to three eclosion days to reach sufficiently high numbers, being fed with flowers and a highly concentrated sucrose solution. Consequently, butterflies were 1-3 days old upon their release into the Metatron (see below).

Metatron setup and experimental design

The Metatron is an experimental platform consisting of interconnected mesh cages ('patches') operated by the 'Station for Theoretical and Experimental Ecology' (SETE) of the CNRS. It allows for the manipulation of spatial and environmental factors in a semi-natural environment (Legrand et al. 2012). Each cage is 200 m^3 (10 x 10 x 2 m) large and is covered by insect-proof gaze. The ground within the cages is covered by natural vegetation. We carried out three experiments in the Metatron as outlined below. Each experiment involved two-patch experimental meta-populations, consisting of a departure and an arrival cage connected by a single, S-shaped corridor (19 m long). To start the experiments, butterflies were released into the departure cage, in which they could either stay or which they could leave through the corridor leading to the arrival cage. Thereafter, butterfly positions were monitored through several recapture sessions (see below). To allow for an appropriate discrimination between dispersers and resident individuals, corridors were designed to be challenging (corridor entries represent less than 2% of the total vertical surface area of patches; Legrand et al. 2012). Moreover, the S-shape of the corridor prevented dispersing butterflies to see the arrival cage. Emigration propensity was scored as the number of butterflies leaving the departure cage, i.e. being re-captured either in the arrival cage or corridor at least once. The number of repeated dispersers, i.e. butterflies moving back and forth between the cages more than once, was low (~ 2%). The vegetation within both cages and the corridors was cut (turf height 10 cm) prior to

172 the start of experiments to allow for the manipulation of host and nectar plant availability (see
173 below).
174
175 In the first two experiments we tested the effect of one environmental factor each, by using 4
176 cages to create 2 two-patch metapopulations per experiment. In experiment 1, we exclusively
177 manipulated resource availability, i.e. one of the departure cages entailed resources (25 pots
178 with flowering nectar plants and additionally 20 pots with host plants) while the other did not.
179 All cages were exposed to full sunlight ('sunny'), conditions suitable for high flight activity.
180 Throughout all experiments, arrival cages had identical conditions with resources and sunny
181 conditions being available, while no resources were available within the corridors. In
182 experiment 2, we exclusively manipulated solar radiation. Here, individuals in both departure
183 cages had access to an equal though limited number of flowering nectar plants (8 pots, no access
184 to host plants), while one departure cage provided sunny and the other one shady and therefore
185 cooler conditions. Shady conditions were achieved through closing motor-driven shutters
186 positioned above the cages (Legrand et al. 2012).
187
188 In the third experiment, we investigated both environmental factors to test for additive /
189 interactive effects. Here, we used in total 8 cages, i.e. 4 two-patch metapopulations. We
190 manipulated resource availability and solar radiation in the departure cages. Two of the
191 departure cages contained resources (25 pots with flowering nectar plants and 20 pots with host
192 plants), while the other two did not. Per resource type, one cage provided sunny and the other
193 one shady conditions. Consequently, we had four types of departure cages: (1) resources and
194 sun, (2) resources and shade, (3) no resources and sun, (4) no resources and shade. Experiments
195 were conducted between 15th and 29th July 2015.
196

We released 138 and 150 into the two departure cages in experiment 1, 92 and 102 in experiment 2, and 107, 108, 113 and 119 butterflies into the 4 cages in experiment 3 (n = 929 in total). Numbers of males and females were equal per cage. In general, *L. tityrus* is a rather sedentary species, which may occur in high local densities in high-quality habitat patches. In experiment 1 and 2 all butterflies were 2 days old upon release into the Metatron, while in experiment 3 age ranged between 1-3 days. The release of butterflies always took place in the morning at 9 a.m. Recapture sessions started in the afternoon (3 p.m.) of the same day and were thereafter performed twice a day (between 9-12 a.m. and 3-6 p.m.; except for rainy conditions). The position (i.e. departure cage, corridor, or arrival cage) of each individual was recorded per session for the following 2.5 (experiments 1 and 2) or 3.5 days (experiment 3). The short observation periods resulted from high mortality within the Metatron due to biotic (predators, e.g. spiders) and abiotic (e.g. heat, thunderstorms) factors (mortality rates: experiment 1 = 68%, experiment 2 = 53%, experiment 3 = 83%). Mortality rates were assessed at the end of each experiment. Butterflies found dead were replaced as long as there were enough freshly eclosed butterflies available. During the last recapture session, all remaining butterflies were caught and subjected to a flight performance test. As *L. tityrus* is a native species in Ariège where the Metatron is located, special care was taken to prevent the escape of butterflies to control the risk of admixture between experimental and wild individuals.

Flight performance test

Butterflies were tested for flight performance using a well-established assay (cf. Ducatez et al. 2012). Each individual was placed into a plastic chamber (30 x 16 x 14 cm), which was perforated at its base and fixed to a rapid agitator (IKA Vortex 4 digital). After a habituation period of 30 seconds, the vortex was switched on to strongly shake the chamber, preventing the butterfly from holding onto the walls. Thus, during the test, butterflies had to fly or were lying

uncomfortably at the bottom of the shaking chamber. The time an individual butterfly spent flying was recorded for 60 seconds, with higher values reflecting a better flight performance. Experiments were performed at 22 ± 0.5°C, mimicking summer (July) conditions in Greifswald. After the performance test, butterflies were frozen in liquid nitrogen for later analyses of potentially flight-related parameters.

Morphological analyses

To test for associations between emigration propensity in the Metatron or flight performance and other traits, we measured several morphological traits. Therefore, frozen butterflies were back-transferred to Greifswald University. First, adult body mass was determined to the nearest 0.01 mg (Sartorius CPA225D). Then, wings, heads and legs were removed. Thorax and abdomen were separated and afterwards weighed. Abdomen fat content was measured following Fischer et al. (2003), but using the less poisonous acetone instead of dichloromethane. Abdomen were first dried to constant weight for two days at 70°C. Abdomen dry mass was measured. Afterwards, fat was extracted twice, each time for 48 h using 1 ml of acetone (C_3H_6O) per butterfly. Solutions were exchanged between both fat extractions. Then, abdomen were again dried for two days at 70°C, after which the fat-free dry mass was measured. Absolute (mg) and relative (%) fat content were determined as the mass difference between abdomen dry mass and the remaining dry mass after the two fat extractions. Forewing area and length (from basis to apex) were measured using digital images of left forewings (captured from ventrally with a digital camera mounted on a stereo microscope) and NIS elements software. Wing loading was calculated as total body mass divided by forewing area, and wing aspect ratio as 4 x forewing length^2 divided by the forewing area (Berwaerts et al. 2002). Thorax-abdomen ratio was calculated by dividing thorax through abdomen mass.

247 *Statistical analyses*

248 Data on morphological traits were analyzed with general linear mixed models (GLMMs) using
249 resources, sun and sex as fixed effects and family as a random effect. In experiment 3, age
250 (fixed factor) was used additionally as covariate (note that experiments 1 and 2 all individuals
251 were 2-days old; see above). Data on individual flight performance and emigration propensity
252 (departure cage left: yes / no) were analyzed using generalized linear models (GzLMs) with
253 either a normal error distribution and a log-link function (flight performance) or a binomial
254 error distribution and logit-link function (emigration propensity), using the same factors as
255 above. Only butterflies where both emigration propensity and flight performance data were
256 available were used for the analyses. All above models were constructed by a stepwise
257 backwards elimination of non-significant factors with p-values > 0.1. Note that an AIC
258 approach yielded identical results.

259

260 To additionally test for the impact of continuous variables (morphology) on flight performance
261 and emigration propensity, we first performed, separately for males and females, principal
262 component analyses (PCAs) owing to strong inter-correlations among traits (Appendix S1:
263 Table S1). The sexes were analyzed separately because GLMM results indicated that males and
264 females differ strongly in their morphological traits (see below). We used the first four principal
265 components (PCs) for further analyses, having Eigenvalues between 4.1 and 0.7 for males and
266 between 5.1 and 1.0 for females (Appendix S1: Table S2). Thus, all PCs explaining ≥ 7% of
267 the variance were included. We then constructed another set of models by including the
268 respective PCs (and flight performance for emigration propensity) as covariates to the models
269 mentioned above, again followed by a stepwise backwards elimination of non-significant
270 factors. All statistical tests were performed with Statistica 12.0 (Tulsa, StatSoft, OK). All means
271 are given ± 1 SE.

272

273

274 # RESULTS

275 ## *Experiment 1*

276 Significant sex differences were found for all morphological traits except from thorax mass,
277 absolute fat content, forewing length, and wing loading (Appendix S1: Table S3), indicating
278 that females had on average higher abdomen and adult body masses, a lower relative fat content,
279 thorax-abdomen ratio and wing aspect ratio, and a larger wing area than males (Table 1). A
280 significant effect of resource availability was found for thorax mass only, which was higher in
281 individuals released into departure cages with resources. However, resource availability was
282 involved in significant interactions with sex for total body mass, abdomen mass, thorax-
283 abdomen ratio, absolute fat content, and wing loading. These interactions indicate that sexual
284 differences were more pronounced in or restricted to individuals being released into cages with
285 resources (Table 1). Differences among families were significant for thorax mass, relative and
286 absolute fat content, wing length, and wing area, and tendencies were found for total body mass
287 and wing aspect ratio.

288

289 Emigration propensity in the Metatron was significantly affected by resource availability only
290 (Table 2a). Individuals with no access to resources showed a higher emigration propensity (38%
291 of 39 vs. 15% of 47; Fig. 1a). Including principal components and flight performance as
292 covariates revealed no significant effects on emigration propensity in either males or females,
293 but the factor resources was still included in both models (Table 2a). Additionally, the factor
294 flight performance was included into the model for males, indicating a non-significant tendency
295 towards a negative correlation between flight performance and emigration propensity (beta -
296 0.11 ± 0.11).

297

298 Flight performance was significantly affected by the factors sex and resource availability (Table
299 2b). Males showed a longer flight endurance than females (36.2 ± 7.6 > 25.6 ± 3.6 s), and
300 individuals that were released into a departure cage with resources flew longer than individuals
301 that were released into a departure cage without resources (38.4 ± 4.7 > 23.4 ± 6.5 s; Fig. 1b).
302 Including principal components as covariates additionally revealed a significant impact of PC2
303 and family for males and PC4 for females (Table 2b). Flight performance was negatively
304 correlated with PC2 in males (beta -0.38 ± 0.22). PC2 in turn was most strongly (positively)
305 correlated with wing area (r = 0.912), wing length (r = 0.865), and thorax mass (r = 0.883;
306 Appendix S1: Table S2a). Thus, smaller males had a better flight performance. Flight
307 performance was negatively correlated with PC4 in females (beta -0.10 ± 0.13). PC4 in turn
308 was most strongly (negatively) correlated with aspect ratio (r = -0.972). Thus, a higher aspect
309 ratio increased flight performance in females (Appendix S1: Table S2b).

310

311 *Experiment 2*

312 Significant sex differences were found for all morphological traits except from thorax mass,
313 relative fat content, and forewing length (Appendix S1: Table S4). Females had on average a
314 higher abdomen and adult body masses, a lower thorax-abdomen ratio, a higher absolute fat
315 content, a larger wing area, a higher wing loading, and a lower wing aspect ratio than males
316 (Table 3). A significant effect of sunny versus shady conditions was found for abdomen mass,
317 thorax-abdomen ratio, and absolute and relative fat content. Individuals that were released into
318 sunny cages had on average a lower abdomen mass, concomitantly a higher thorax-abdomen
319 ratio, and a lower absolute and relative fat content. Furthermore, significant sun by sex
320 interactions were found for abdomen mass, thorax-abdomen ratio, relative fat content, and wing
321 loading (Appendix S1: Table S4). They show that sexual differences were more pronounced

under sunny as compared with shady conditions, except for relative fat content (Table 3). Differences among families were significant for thorax mass, wing length, and wing area.

Emigration propensity was significantly affected by sunny versus shady conditions only (Table 4a). Individuals showed a higher emigration propensity when released into a shady rather than sunny departure cage (36.7% of 49 > 4.8% of 42; Fig. 2a). This pattern also prevailed in females when being analyzed separately. Including principal components and flight performance as covariates did not change the above results, as no covariate was included within the final models.

Flight performance was not affected by the factors sex, sun or family in the first model. Including covariates revealed no significant influence of any factors on females, but a significant impact of PC1 to 4 on males. Additionally, sun and family tended to influence flight performance in males (Table 4b). Males that were released into shady departure cages tended to fly marginally longer than those released into sunny departure cages (33.1 ± 8.6 > 30.3 ± 7.4 s; Fig. 2b). Male flight performance was negatively correlated with PC1 (beta -0.27 ± 0.27), PC2 (beta -0.05 ± 0.26) and PC4 (beta -0.05 ± 0.30) and positively with PC3 (beta 0.10 ± 0.30). PC1 in turn was most strongly (positively) correlated with abdomen mass (r = 0.953), PC2 most strongly (positively) with wing area (r = 0.912), PC3 and PC4 most strongly (positively) with aspect ratio (PC3: r = 0.743, PC4: r = 0.563; Appendix S1: Table S2a). Thus, males with a lower abdomen mass and thus wing loading and a smaller wing area showed a better flight performance, while aspect ratio showed no clear association with flight performance.

345 *Experiment 3*

346 Significant sex differences were found for all morphological traits measured except from thorax
347 mass, relative fat content, and forewing length (Appendix S1: Table S5), indicating that females
348 had on average higher abdomen and total body masses, a lower thorax-abdomen ratio, a higher
349 absolute fat content (mg), a larger wing area, a higher wing loading, and a lower wing aspect
350 ratio than males (Table 5). A significant effect of resource availability was found for relative
351 and absolute fat content only, showing that individuals that had access to resources had a lower
352 relative (7.9 ± 0.6% vs. 15.1 ± 1.4%) and absolute (0.31 ± 0.03 mg vs. 0.70 ± 0.08 mg) abdomen
353 fat content. Effects of sunny versus shady conditions, in contrast, were non-significant
354 throughout, as was the case for all interaction terms. Differences among families were
355 significant for wing aspect ratio only. A significant effect of age was found for wing area only,
356 indicating that older butterflies had larger wings than younger ones (thus, largest butterflies
357 eclosed first).
358
359 Emigration propensity was significantly affected by the factors resource availability, the
360 interaction between resources and sex, and the three-way interaction with sun (Table 6a). Thus,
361 individuals that were released into departure cages without resources showed a higher
362 emigration propensity (46% of 28 > 24% of 45). The resources by sex interaction shows that
363 females had a higher emigration propensity than males if resources were present in the departure
364 cage, while it was the other way round if no resources were present (Fig. 3a). Consequently,
365 emigration propensity increased much more strongly in males than in females when no
366 resources were present. The significant three-way interaction additionally shows that males
367 were more dispersive than females only if a lack of resources coincided with sunny conditions
368 (60% of 5 vs. 22% of 9). Under all other conditions, females were the more dispersive sex
369 (resources + sun: 60% of 10 vs. 0% of 7; resources + shade: 22% of 18 vs. 10% of 10; no

370 resources + shade: 58% of 12 vs. 50% of 2). Including principal components and flight
371 performance as covariates revealed an effect of the interaction between resources and sun for
372 females and PC3 for males (Table 6a). The resources by sun interaction for females shows that
373 females had a higher emigration propensity if resources and sun were present (60% of 10) or
374 both absent (58% of 12) in the departure cage, while emigration propensity was lower when
375 one of the factors was absent (resources + shade: 22% of 18; no resources + sun: 22% of 9). In
376 males, PC3 showed a positive correlation with emigration propensity (beta 0.50 ± 0.19). PC3
377 in turn was most strongly (positively) correlated with aspect ratio (r = 0.743), indicating that
378 emigration propensity was positively associated with aspect ratio in males (Appendix S1: Table
379 S2a).
380
381 Flight performance in the vortex was significantly affected by the factors sex, sun, and family
382 (Table 6b). Males showed a better flight performance than females (38.3 ± 7.0 > 25.6 ± 4.8 s),
383 and individuals that were released into shady departure cages flew longer than those released
384 into sunny departure cages (39.0 ± 5.3 > 24.9 ± 6.5 s; Fig. 3b). Additionally including principal
385 components as covariates revealed a significant impact of PC1 for females and PC3 for males.
386 Flight performance was negatively correlated with PC3 in males (beta -0.24 ± 0.22). PC3 in
387 males was most strongly (positively) correlated with aspect ratio (r = 0.743, Appendix S1: Table
388 S2a), meaning that flight performance was negatively correlated with aspect ratio in males.
389 Flight performance was negatively correlated with PC1 in females (beta -0.27 ± 0.14). PC1 in
390 females was in turn most strongly (negatively) correlated with body mass (r = -0.974; Appendix
391 S1: Table S2b). Thus, flight performance was positively related to body mass in females.
392
393

DISCUSSION

We start our discussion with variation in morphological traits, as these have been implied to affect flight ability in insects and may thus underlie variation in emigration propensity.

Sexual differences in morphological traits

The morphological traits measured were, as expected, strongly affected by sex. Our results showed consistently that females were heavier than males, which is usually explained by fecundity selection for large body size in females, as both traits are often positively related (Honek 1993, Blanckenhorn 2000). Male insects, in contrast, often favor early adult emergence to maximize mating opportunities, resulting in rapid development which is in turn traded off against body size (selection for protandry; Wiklund and Fagerström 1977, Karl and Fischer 2008). In accordance with above, mass differences between the sexes were caused by pronounced variation in abdomen mass, enabling high fecundity and large amounts of storage reserves to fuel egg production in females (Eaton 1988, Tammaru et al. 1996, Berwaerts et al. 2002). Note here the higher absolute fat content found in females, at least under favorable conditions, while relative fat content is typically higher in males in order to fuel flight activity (Zera et al. 1998, Karl and Fischer 2008).

The females' higher mass results in a higher wing loading compared with males, which is likely detrimental to flight performance (Wickman 2009, Saastamoinen et al. 2012). Males and females also differed clearly in wing morphology. Despite similar wing lengths, females showed larger wing areas resulting from having wider wings, while those of males are more elongated as evidenced by a higher wing aspect ratio (Dudley 2000, Hassall 2015). Thorax mass, in contrast, did not differ among sexes, demonstrating that males have relatively larger thoraxes than females, resulting in a consistently increased thorax-abdomen ratio (Berwaerts et

al. 2002, Karl et al. 2008). The above sex-specific differences in morphology may result from differential selective pressures favoring excellent flight performance to succeed in competition for mates in males (Van Dyck and Wiklund 2002, Berwaerts et al. 2006).

Environmental differences in morphological traits

As expected for a study carried out under semi-natural conditions, effects of environmental conditions were generally weaker and less consistent than sexual differences. Storage reserves, namely fat content, were reduced if resources were present in the departure cages in experiment 3. We assume that this counterintuitive result stems from butterflies remaining largely inactive when being deprived of food in order to save energy and to endure the critical phase (Saastamoinen and Hanski 2008). Furthermore, females need nectar for egg production in copper butterflies, without which less energy will be allocated to reproduction (Arrese and Soulages 2010, Karl et al. 2011). Both mechanisms result in a reduced consumption of storage reserves, at least initially (note here the young age of the butterflies tested). The above interpretation of an important impact of activity on storage reserves is further supported by the fact that the highest mean fat content was found in individuals being deprived of food under shady conditions, as a lack of solar radiation will certainly further reduce butterfly activity (Wickman 2009, Kuussaari et al. 2016). Furthermore, experiment 2 yielded identical results, as individuals released into sunny cages had on average a lower abdomen mass, a higher thorax-abdomen ratio, and a lower fat content. However, in experiment 1 the above patterns did not prevail, for which we have no explanation.

In experiments 1 and 2, sexual differences were more pronounced when individuals had access to food or when they were exposed to sunny conditions. The former indicates that females gained more mass when having access to food, probably as a result of egg maturation (Karl et

al. 2011). The latter indicates that males lost more mass and fat under sunny conditions than females, which may reflect their high flight activity (see above) under such beneficial conditions.

Effects on emigration propensity

A lack of resources needed for adult feeding and oviposition caused, as expected, a higher emigration propensity. Thus, individuals apparently showed a higher motivation to leave the departure patch in search for vital resources. Also, emigration propensity was higher under shady as compared with sunny conditions. This is intriguing since shade / lack of opportunity for sun basking will certainly reduce overall flight activity (e.g. Fischer and Fiedler 2001). Butterflies require high muscle temperatures between 30-38°C for flight activity, which is achieved through basking in the sun (e.g. Watt 1995, Wickman 2009). Taken together, these findings clearly suggest that emigration in *L. tityrus* is not merely driven by chance effects, but that butterflies can rather accurately assess patch quality and actively decide to leave a patch in case of degraded habitat quality. Such conditional, context-dependent behavior allows to escape from patches of unfavorable quality (Matthysen 2012). Accordingly, directed flight has been observed to occur more often in unsuitable habitats (Dennis 2004).

Overall, females were the more dispersive sex in *L. tityrus*. In experiment 3, females showed a clearly higher emigration propensity than males if resources were present. Several reasons may explain why females should indeed be more dispersive than males: (1) single mated females are, in contrast to males, able to found a new population, (2) females may emigrate more readily to escape from male harassment or as a risk spreading strategy by distributing their eggs over a wider area (Hill et al. 1999, Hopper 1999, Trochet et al. 2013). When being confronted with a lack of resources, both sexes increased emigration rates. However, this increase was much more

pronounced in males, causing an even higher emigration propensity under such conditions than in females. This is likely caused by males searching for appropriate sites to establish a territory, which typically cover dense patches of flowering nectar plants (Ebert and Rennwald 1991, Fischer and Fiedler 2001). The fact that male emigration was most strongly promoted when a lack of resources coincided with sunny conditions probably indicates that males took advantage of beneficial conditions for flight to search for territories. However, sex differences were not significant in experiments 1 and 2, but note that the principal pattern described above prevailed also in experiment 2 (not attaining significance) and to some extent even in experiment 1.

The impact of condition on emigration propensity though was remarkably low. Only in one out of 6 of our analyses (males in experiment 3), a PC (PC3) had a significant impact, indicating a positive association between wing aspect ratio and emigration propensity. Note that flight performance was only once included into the final models (though not attaining significance), reinforcing the above notion that emigration in *L. tityrus* is in the first place governed by motivation (i.e. context) rather than flight ability.

Variation in flight performance

In two out of three experiments, males showed better flight performance than females. We cannot explain the results obtained in experiment 2, but assume that males indeed typically show a better flight performance than females. This would be in line with the above morphological differences between sexes. Furthermore, individuals released into shady compared with sunny departure cages had a better flight performance. Thus, conditions promoting flight activity diminish subsequent flight performance, presumably as a result of exhaustion and resource depletion. This is further supported by results from experiment 1, in which a lack of resources had a negative effect on flight performance.

Morphological traits had apparently a larger impact on flight performance than on emigration propensity (significant impact of PCs in 5 out of 6 analyses). This suggests that differences in flight performance between males and females can indeed be readily explained by morphological differences as has been repeatedly suggested (Van Dyck and Wiklund 2002, Berwaerts et al. 2006). In all three experiments, at least one PC turned out to be significant for males. Accordingly, smaller (lighter) males and those having a lower wing loading had a better flight performance. Additionally, lower abdomen masses increase the thorax-abdomen-ratio, which has often been found to enhance flight performance (see above). Wing aspect ratio, in contrast, showed no consistent association with flight performance. Regarding females, one PC turned out to be significant in two of the experiments. Flight performance was positively related to aspect ratio (experiment 1) and body mass (experiment 3). Here, a high body mass (with an accordingly high fat content to fuel flight) may reflect an overall good condition.

Conclusions

Despite problems with high mortality restricting our observations to the first days of adult life, our results show some clear patterns which thus likely bear biological significance. Note that adult life span in copper butterflies is relatively short, typically ranging between 8-10 days on average in nature (Fischer et al. 1999). Based on experiments performed under semi-natural conditions, we obtained some interesting results. First, males and females differed substantially in morphology, with males showing traits typically associated with better flight performance, which we could verify in a flight performance test. Such differences most likely result from selection on males for increased acceleration speed, maneuverability and endurance in order to succeed in aerial combats with rivalling males and ultimately competition for females (Saastamoinen et al. 2012). Second and more interestingly, the traits underlying increased flight

519 performance (i.e. condition) did not affect emigration propensity. For instance, although
520 females showed traits associated with diminished flight performance, they showed a higher
521 emigration propensity than males (when resources were present), which can be readily
522 explained in ecological and evolutionary contexts. This indicates that flight performance and
523 the first step of dispersal, i.e. the actual decision whether to disperse or not, do not necessarily
524 equate, casting doubt on the intuitive assumption of a general, positive relationship between the
525 two. Note that the study design did not allow to examine the other stages of dispersal. Thus, we
526 cannot assess the effects of flight performance on dispersal success. Third, environmental
527 conditions (i.e. the context) affected condition, flight performance, but also emigration
528 propensity. Notably, conditions indicative of poor habitat quality such as shade and a lack of
529 resources promoted emigration. This suggests that emigration in *L. tityrus* is not merely a
530 random process, but is governed by an active decision in a context-dependent manner,
531 following an accurate assessment of patch quality. In summary, our results suggest that
532 emigration propensity in this butterfly is a highly plastic, context-dependent trait triggered
533 largely by habitat quality rather than by individual condition (Saastamoinen et al. 2012, Legrand
534 et al. 2015). Besides abiotic factors, resource availability, and harassment, this may also involve
535 density dependence as has been previously shown in other studies (Enfjäll and Leimar 2005,
536 Trochet et al. 2013). Importantly, deteriorating habitat quality promotes emigration propensity
537 (Legrand et al. 2015), which should be considered when trying to forecast future species
538 distribution. For instance, climate change is likely to reduce habitat quality, but this will at the
539 same time likely increase emigration propensity and thereby potentially facilitate range shifts
540 in flying insects (Travis et al. 2013).
541
542

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700

701 TABLES

702 TABLE 1. Morphological traits of *Lycaena tityrus* in relation to sex and the presence of
703 resources in the departure cage. Given are means ± 1 SE. Group sample sizes are 16 for fed
704 males, 7 for unfed males, 31 for fed females and 34 for unfed females. Different superscript
705 letters indicate significant differences among groups.

706

	Resources		**No resources**	
Trait	**Males**	**Females**	**Males**	**Females**
Adult body mass (mg)	29.2 ± 0.98^{a}	37.3 ± 1.20^{b}	28.1 ± 3.04^{a}	32.4 ± 1.45^{a}
Thorax mass (mg)	15.2 ± 0.62^{a}	14.3 ± 0.40^{a}	12.6 ± 1.10^{a}	13.2 ± 0.57^{a}
Abdomen mass (mg)	7.9 ± 0.76^{a}	16.7 ± 0.71^{b}	10.3 ± 2.71^{ac}	13.3 ± 0.82^{c}
Thorax-abdomen ratio	2.1 ± 0.20^{a}	0.9 ± 0.03^{b}	1.7 ± 0.34^{a}	1.0 ± 0.04^{b}
Fat (mg)	0.4 ± 0.10^{ab}	0.5 ± 0.04^{b}	0.3 ± 0.16^{ab}	0.3 ± 0.04^{a}
Fat (%)	13.3 ± 2.67^{a}	8.8 ± 0.60^{ab}	12.9 ± 4.27^{a}	5.5 ± 0.56^{b}
Wing length (mm)	14.7 ± 0.17^{a}	14.8 ± 0.13^{a}	14.2 ± 0.39^{a}	14.9 ± 0.14^{a}
Wing area (mm^2)	80.8 ± 1.85^{a}	88.1 ± 1.51^{b}	75.7 ± 4.20^{a}	89.1 ± 1.62^{b}
Wing loading	36.2 ± 1.08^{a}	42.3 ± 1.01^{b}	37.7 ± 4.55^{ab}	36.1 ± 1.21^{a}
Wing aspect ratio	10.7 ± 0.10^{a}	10.0 ± 0.05^{b}	10.7 ± 0.13^{a}	10.0 ± 0.05^{b}

707

TABLE 2. Results of generalized linear models for the effects of resources, sex, and family on (a) emigration propensity (logit-link function and binomial error distribution) and (b) flight performance (flight duration in a vortex; log-link function and normal error distribution) in the butterfly *Lycaena tityrus* (models 1). Second models were constructed separately for males and females by additionally including principal components (PCs) as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. Significant p-values are given in bold.

TABLE 2a

Model 1	**d.f.**	**Odds ratio**	**95% CI**	**Wald Chi^2**	**p**
Resources	1	0.280	0.010-0.784	5.9	**0.0154**
Model 2 Males	**d.f.**	**Odds ratio**	**95% CI**	**Wald Chi^2**	**p**
Flight performance	1	1.121	0.985-1.276	3.0	0.0837
Resources	1	0.005	< 0.001-1.674	3.2	0.0737
Model 2 Females	**d.f.**	**Odds ratio**	**95% CI**	**Wald Chi^2**	**p**
Resources	1	0.360	0.107-1.215	2.7	0.0996

718 TABLE 2b

Model 1	**d.f.**	**Parameter estimate**	**SE**	**Wald Chi^2**	**p**
Resources	1	-0.25	0.09	8.7	**0.0032**
Sex	1	0.18	0.07	6.5	**0.0109**
Family	6			11.3	0.0808
Model 2 Males	**d.f.**	**Parameter estimate**	**SE**	**Wald Chi^2**	**p**
Resources	1	-0.24	0.10	5.5	**0.0195**
PC2	1	-0.26	0.08	10.8	**0.0010**
Family	5			21.0	**0.0008**
Model 2 Females	**d.f.**	**Parameter estimate**	**SE**	**Wald Chi^2**	**p**
Resources	1	-0.43	0.13	11.3	**0.0008**
PC4	1	-0.16	0.08	3.8	**0.0498**

719

720 TABLE 3. Morphological traits of *Lycaena tityrus* in relation to sex and sunny versus shady
721 conditions in the departure cage. Given are means ± 1 SE. Group sample size is 11 for males
722 and 31 for females at sunny, and 8 for males and 31 for females at shady conditions. Different
723 superscript letters indicate significant differences among groups.
724

	Sun		Shade	
Trait	**Males**	**Females**	**Males**	**Females**
Adult body mass (mg)	27.8 ± 0.61^{a}	40.0 ± 1.53^{b}	32.2 ± 2.68^{ab}	39.4 ± 1.94^{b}
Thorax mass (mg)	14.9 ± 0.49^{a}	14.8 ± 0.54^{a}	13.7 ± 0.90^{a}	14.3 ± 0.59^{a}
Abdomen mass (mg)	7.0 ± 0.74^{a}	19.2 ± 1.00^{b}	13.0 ± 2.28^{ab}	19.2 ± 1.32^{b}
Thorax-abdomen ratio	2.3 ± 0.22^{a}	0.8 ± 0.03^{b}	1.4 ± 0.30^{c}	0.8 ± 0.05^{b}
Fat (mg)	0.2 ± 0.07^{a}	1.0 ± 0.15^{b}	1.2 ± 0.26^{b}	1.1 ± 0.11^{b}
Fat (%)	11.6 ± 2.86^{a}	15.2 ± 1.43^{a}	29.1 ± 3.24^{b}	18.4 ± 1.30^{a}
Wing length (mm)	14.9 ± 0.14^{a}	14.8 ± 0.12^{a}	14.5 ± 0.15^{a}	14.8 ± 0.13^{a}
Wing area (mm^2)	82.9 ± 1.49^{ab}	88.5 ± 1.64^{b}	77.4 ± 1.66^{a}	88.8 ± 1.57^{b}
Wing loading	33.7 ± 1.08^{a}	45.0 ± 1.30^{b}	41.5 ± 2.97^{ab}	43.8 ± 1.65^{b}
Wing aspect ratio	10.8 ± 0.06^{a}	9.9 ± 0.03^{b}	10.9 ± 0.12^{a}	9.9 ± 0.05^{b}

725

TABLE 4. Results of generalized linear models for the effects of sun, sex and family on (a) emigration propensity (logit-link function and binomial error distribution) and (b) flight performance (flight duration in a vortex; log-link function and normal error distribution) in the butterfly *Lycaena tityrus* (model 1). Second models were constructed separately for males and females by additionally including principal components (PCs) as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. For (a), no PC was included for females and no factor at all was selected for males within the second models. For (b), no factor was selected in model 1 and in model 2 for females. Significant p-values are given in bold.

TABLE 4a

Model 1	**d.f.**	**Odds ratio**	**95% CI**	**Wald Chi^2**	**p**
Sun	1	0.086	0.019-0.399	9.8	**0.0017**
Model 2 Females	**d.f.**	**Odds ratio**	**95% CI**	**Wald Chi^2**	**p**
Sun	1	0.111	0.023-0.544	7.4	**0.0067**

738 TABLE 4b

Model 2 Males	**d.f.**	**Parameter estimate**	**SE**	**Wald Chi^2**	**p**
PC1	1	-0.37	0.16	5.6	**0.0181**
PC2	1	-0.43	0.22	4.1	**0.0437**
PC3	1	0.68	0.28	6.3	**0.0122**
PC4	1	0.88	0.42	4.5	**0.0343**
Sun	1	0.33	0.20	2.8	0.0956
Family	5			9.3	0.0980

739

740 TABLE 5. Morphological traits of *Lycaena tityrus* in relation to sex. Given are means ± 1 SE.
741 Group sample size is 24 for males and 49 for females. Different superscript letters indicate
742 significant differences among groups.

743

Trait	**Males**	**Females**
Adult body mass (mg)	27.9 ± 0.98^{a}	37.2 ± 1.31^{b}
Thorax mass (mg)	14.9 ± 0.06^{a}	14.3 ± 0.04^{a}
Abdomen mass (mg)	7.1 ± 0.04^{a}	16.9 ± 0.09^{b}
Thorax-abdomen ratio	2.3 ± 0.14^{a}	0.9 ± 0.04^{b}
Fat (mg)	0.2 ± 0.04^{a}	0.6 ± 0.06^{b}
Fat (%)	11.2 ± 1.41^{a}	10.5 ± 0.91^{b}
Wing length (mm)	14.5 ± 0.16^{a}	14.8 ± 0.11^{a}
Wing area (mm^2)	78.5 ± 1.70^{a}	86.8 ± 1.19^{b}
Wing loading	35.6 ± 0.87^{a}	42.3 ± 1.35^{b}
Wing aspect ratio	10.8 ± 0.07^{a}	9.9 ± 0.04^{b}

744

TABLE 6. Results of generalized linear models for the effects of resources, sun, sex, family and age on (a) emigration propensity (logit-link function and binomial error distribution) and (b) flight performance (flight duration in a vortex; log-link function and normal error distribution) in the butterfly *Lycaena tityrus* (models 1). Second models were constructed separately for males and females by additionally including principal components (PCs) as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. Significant p-values are given in bold.

TABLE 6a

Model 1	**Odds ratio**	**95% CI**	**d.f.**	**Wald Chi^2**	**p**
Resources	0.222	0.061-0.811	1	5.2	**0.0227**
Resources * Sex			1	5.3	**0.0217**
Resources * Sun * Sex			1	5.6	**0.0183**
Model 2 Males	**Odds ratio**	**95% CI**	**d.f.**	**Wald Chi^2**	**p**
PC3	0.259	0.067-1.003	1	3.8	0.0505
Model 2 Females	**Odds ratio**	**95% CI**	**d.f.**	**Wald Chi^2**	**p**
Resources * Sun			1	5.1	**0.0243**

755 TABLE 6b

Model 1	**Parameter estimate**	**SE**	**d.f.**	**Wald Chi^2**	**p**
Sun	0.22	0.09	1	5.9	**0.0153**
Sex	0.28	0.09	1	8.9	**0.0028**
Family			5	12.1	**0.0341**
Model 2 Males	**Parameter estimate**	**SE**	**d.f.**	**Wald Chi^2**	**p**
PC3	-0.62	0.28	1	4.9	**0.0276**
Family			4	8.4	0.0794
Model 2 Females	**Parameter estimate**	**SE**	**d.f.**	**Wald Chi^2**	**p**
PC1	-0.10	0.05	1	3.9	**0.0470**

756

757 FIGURE LEGENDS

758

759 FIGURE 1. Emigration propensity (%, a) and flight performance (means + 1 SE, b) of *Lycaena*
760 *tityrus* in relation to the presence or absence of resources in the departure cage. Males: filled
761 bars; females: open bars.

762

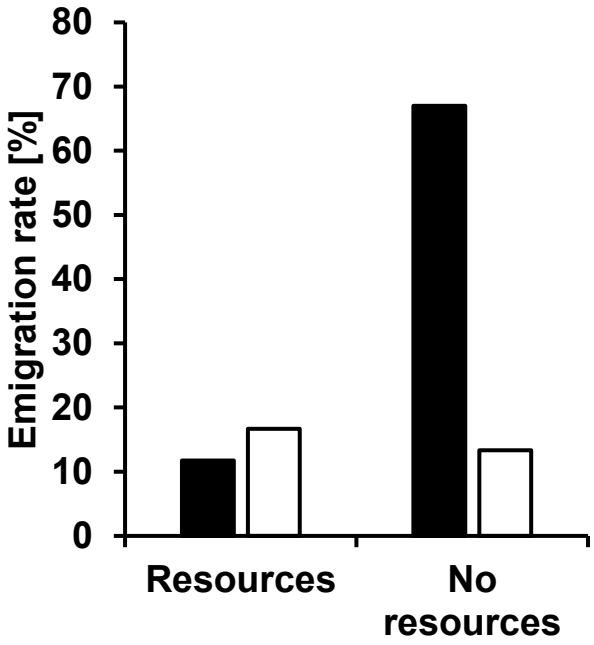
763 FIGURE 2. Emigration propensity (%, a) and flight performance (means + 1 SE, b) of *Lycaena*
764 *tityrus* in relation to having experienced sunny or shady conditions in the departure cage. Males:
765 filled bars; females: open bars.

766

767 FIGURE 3. Emigration propensity (%, a) in relation to the presence or absence of resources
768 and flight performance (means + 1 SE, b) in relation to having experienced sunny or shady
769 conditions in the departure cage in *Lycaena tityrus*. Males: filled bars; females: open bars.

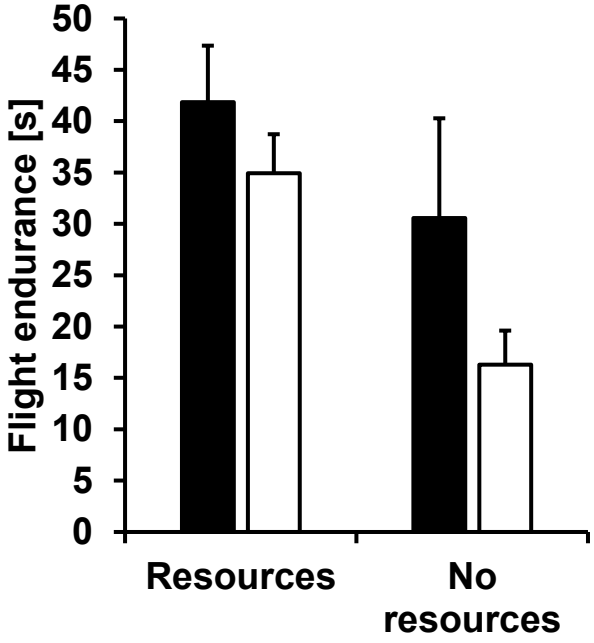
770

771 FIG. 1a



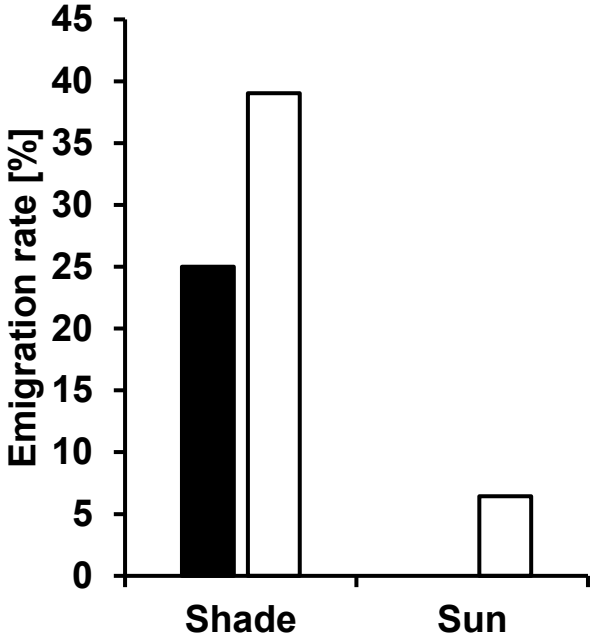
772

773 FIG. 1b



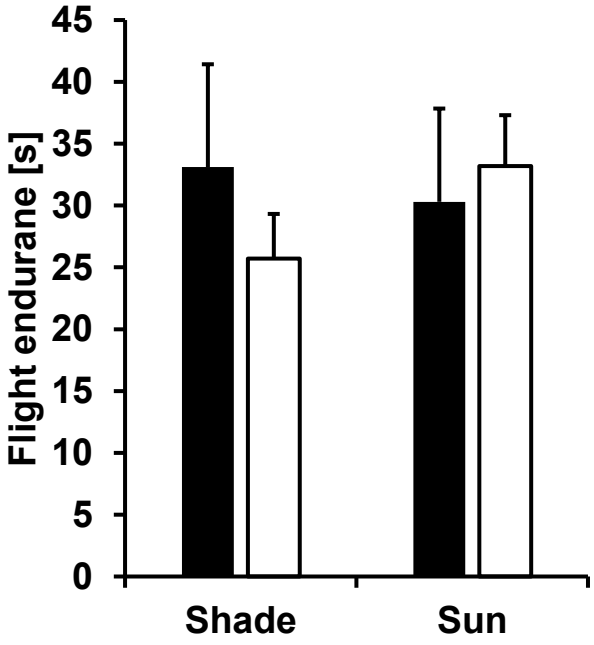
774

775 FIG. 2a



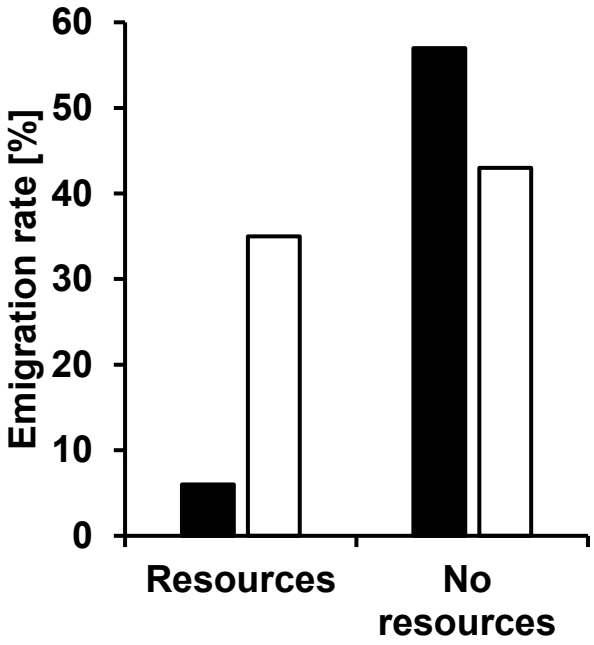
776

777 FIG. 2b



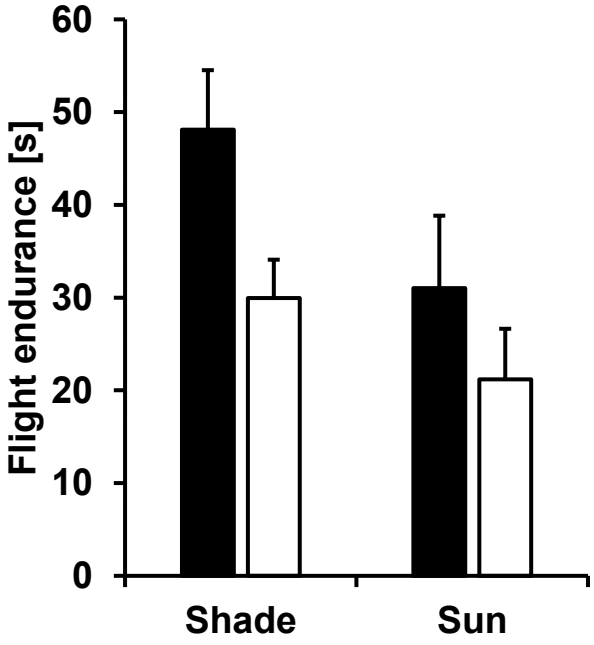
778

779 FIG. 3a



780

781 FIG. 3b



782

APPENDIX S1

TABLE S1. Correlations among morphological parameters in *L. tityrus* for experiment 1 (a), 2 (b) and 3 (c). Significant p-values are given in bold.

TABLE S1a

	Adult body mass	Abdomen mass	Thorax mass	Fat (mg)	Fat (%)	Wing length	Wing area	Thorax-abdomen ratio	Wing loading	Wing aspect ratio
Adult body mass	1.000	**0.904**	**0.742**	**0.656**	**0.239**	**0.549**	**0.614**	**-0.439**	**0.869**	**-0.296**
Abdomen mass	**0.904**	1.000	**0.394**	**0.655**	0.185	**0.316**	**0.438**	**-0.740**	**0.859**	**-0.383**
Thorax mass	**0.742**	**0.394**	1.000	**0.404**	**0.266**	**0.647**	**0.591**	0.214	**0.561**	-0.017
Fat (mg)	**0.656**	**0.655**	**0.404**	1.000	**0.791**	**0.221**	**0.231**	**-0.357**	**0.686**	-0.067
Fat (%)	**0.239**	0.185	**0.266**	**0.791**	1.000	0.011	-0.114	0.083	**0.396**	**0.341**
Wing length	**0.549**	**0.316**	**0.647**	**0.221**	0.010	1.000	**0.930**	0.059	0.120	-0.073
Wing area	**0.614**	**0.438**	**0.591**	**0.232**	-0.114	**0.930**	1.000	-0.129	0.153	**-0.431**
Thorax-abdomen ratio	**-0.439**	**-0.740**	0.214	**-0.357**	0.083	0.059	-0.129	1.000	**-0.477**	**0.470**
Wing loading	**0.869**	**0.859**	**0.561**	**0.686**	**0.396**	0.121	0.153	**-0.477**	1.000	-0.097
Wing aspect ratio	**-0.296**	**-0.383**	-0.017	-0.067	**0.341**	-0.073	**-0.431**	**0.470**	-0.097	1.000

TABLE S1b

	Adult body mass	Abdomen mass	Thorax mass	Fat (mg)	Fat (%)	Wing length	Wing area	Thorax-abdomen ratio	Wing loading	Wing aspect ratio
Adult body mass	1.000	**0.946**	**0.737**	**0.456**	0.088	**0.581**	**0.727**	**-0.546**	**0.933**	**-0.493**
Abdomen mass	**0.946**	1.000	**0.484**	**0.543**	0.187	**0.436**	**0.638**	**-0.742**	**0.906**	**-0.570**
Thorax mass	**0.737**	**0.485**	1.000	0.118	-0.139	**0.658**	**0.637**	0.059	**0.640**	-0.155
Fat (mg)	**0.456**	**0.543**	0.118	1.000	**0.753**	**0.270**	**0.327**	**-0.482**	**0.427**	-0.197
Fat (%)	0.088	0.187	-0.139	**0.753**	1.000	0.075	0.001	-0.190	0.104	0.173
Wing length	**0.581**	**0.436**	**0.658**	**0.270**	0.075	1.000	**0.896**	< 0.001	**0.301**	-0.083
Wing area	**0.727**	**0.638**	**0.637**	**0.327**	0.001	**0.896**	1.000	**-0.274**	**0.438**	**-0.514**
Thorax-abdomen ratio	**-0.546**	**-0.742**	0.059	**-0.482**	-0.190	< 0.001	**-0.274**	1.000	**-0.583**	**0.603**
Wing loading	**0.933**	**0.906**	**0.640**	**0.427**	0.104	**0.301**	**0.438**	**-0.583**	1.000	**-0.383**
Wing aspect ratio	**-0.493**	**-0.570**	-0.155	-0.197	0.173	-0.083	**-0.514**	**0.603**	**-0.383**	1.000

TABLE S1c

	Adult body mass	Abdomen mass	Thorax mass	Fat (mg)	Fat (%)	Wing length	Wing area	Thorax-abdomen ratio	Wing loading	Wing aspect ratio
Adult body mass	1.000	**0.917**	**0.665**	**0.534**	0.038	**0.454**	**0.589**	**-0.521**	**0.927**	**-0.379**
Abdomen mass	**0.917**	1.000	**0.319**	**0.598**	0.076	**0.261**	**0.455**	**-0.762**	**0.891**	**-0.479**
Thorax mass	**0.665**	**0.319**	1.000	0.145	-0.045	**0.540**	**0.495**	0.183	**0.567**	0.009
Fat (mg)	**0.534**	**0.598**	0.145	1.000	**0.699**	0.134	0.225	**-0.464**	**0.542**	**-0.236**
Fat (%)	0.038	0.076	-0.045	**0.699**	1.000	-0.144	-0.208	-0.013	0.139	0.163
Wing length	**0.454**	**0.261**	**0.540**	0.134	-0.144	1.000	**0.893**	0.002	0.135	0.056
Wing area	**0.589**	**0.455**	**0.495**	0.225	-0.208	**0.893**	1.000	**-0.254**	**0.249**	**-0.396**
Thorax-abdomen ratio	**-0.521**	**-0.762**	0.183	**-0.464**	-0.013	0.002	**-0.254**	1.000	**-0.517**	**0.571**
Wing loading	**0.927**	**0.891**	**0.567**	**0.543**	0.139	0.135	**0.249**	**-0.517**	1.000	**-0.277**
Wing aspect ratio	**-0.379**	**-0.479**	0.009	**-0.236**	0.163	0.056	**-0.396**	**0.571**	**-0.277**	1.000

TABLE S2. Principal component analysis (PCA) results for males (a) and females (b) including Eigenvalues, percentage of the variance explained, cumulative percentage of the variance explained, and r-values of correlations with continuous variables (rows 4-13) for principal components 1-4. r-values > 0.7 in bold.

TABLE S2a

No.	Parameter	PC1	PC2	PC3	PC4
1	Eigenvalue	4.1	3.0	1.5	0.7
2	Variance explained (%)	41.2	30.0	15.2	7.0
3	Cumulative variance (%)	41.2	71.2	86.4	93.4
4	Adult body mass	0.697	0.624	-0.320	0.126
5	Thorax mass	-0.151	**0.883**	-0.256	0.052
6	Abdomen mass	**0.953**	0.052	-0.174	0.096
7	Thorax-abdomen ratio	**-0.818**	0.354	0.071	-0.211
8	Fat (mg)	**0.811**	0.157	0.416	-0.333
9	Fat (%)	0.687	0.210	0.536	-0.388
10	Wing length	-0.198	**0.865**	0.359	0.184
11	Wing area	-0.264	**0.912**	0.124	0.007
12	Wing loading	**0.869**	0.134	-0.397	0.135
13	Wing aspect ratio	0.203	-0.188	**0.743**	0.563

TABLE S2b

No.	Parameter	PC1	PC2	PC3	PC4
1	Eigenvalue	5.1	2.0	1.1	1.0
2	Variance explained (%)	50.7	20.2	11.1	10.2
3	Cumulative variance (%)	50.7	70.9	82.0	92.2
4	Adult body mass	**-0.974**	0.094	0.180	-0.041
5	Thorax mass	**-0.796**	0.399	0.112	-0.030
6	Abdomen mass	**-0.952**	-0.129	0.212	-0.038
7	Thorax-abdomen ratio	0.628	0.491	-0.271	0.055
8	Fat (mg)	-0.636	-0.534	-0.495	0.058
9	Fat (%)	-0.427	-0.699	-0.509	0.072
10	Wing length	-0.617	0.634	-0.393	-0.130
11	Wing area	-0.656	0.623	-0.342	0.149
12	Wing loading	**-0.874**	-0.166	0.385	-0.133
13	Wing aspect ratio	0.171	-0.033	-0.155	**-0.972**

TABLE S3. Results of general linear mixed models for the effects of resources, sex (fixed factors), and family (random) on morphological traits in *Lycaena tityrus* for individuals released in experiment 1. Models were constructed using a stepwise backwards elimination of non-significant factors. Significant p-values are given in bold.

Adult body mass	**MS**	**d.f.**	**F**	**p**
Sex	$3.9 * 10^{-4}$	1	8.9	**0.0037**
Resources * Sex	$1.4 * 10^{-4}$	2	3.1	**0.0493**
Family	$0.9 * 10^{-4}$	6	2.1	0.0595
Error	$0.4 * 10^{-4}$	78		
Thorax mass	**MS**	**d.f.**	**F**	**p**
Resources	$0.31 * 10^{-4}$	1	5.0	**0.0275**
Family	$0.22 * 10^{-4}$	6	3.5	**0.0040**
Error	$0.06 * 10^{-4}$	80		
Abdomen mass	**MS**	**d.f.**	**F**	**p**
Sex	$5.6 * 10^{-4}$	1	30.6	**<0.0001**
Resources * Sex	$2.2 * 10^{-4}$	1	11.9	**0.0009**
Error	$0.2 * 10^{-4}$	85		
Thorax-abdomen ratio	**MS**	**d.f.**	**F**	**p**
Sex	14.9	1	86.0	**<0.0001**
Resources * Sex	0.6	2	3.5	**0.0357**
Family	0.3	6	1.8	0.1076
Error	0.2	78		

Table S3 continued

Fat (mg)	**MS**	**d.f.**	**F**	**p**
Resources * Sex	0.31	3	4.4	**0.0063**
Family	0.20	6	2.8	**0.0151**
Error	0.07	78		
Fat (%)	**MS**	**d.f.**	**F**	**p**
Sex	373	1	12.8	**0.0006**
Resources * Sex	77	2	2.7	0.0767
Family	114	6	3.9	**0.0018**
Error	29	78		
Wing length	**MS**	**d.f.**	**F**	**p**
Family	3.6	6	10.3	**<0.0001**
Error	0.3	77		
Wing area	**MS**	**d.f.**	**F**	**p**
Sex	855	1	20.4	**<0.0001**
Family	496	6	11.8	**<0.0001**
Error	42	76		
Wing loading	**MS**	**d.f.**	**F**	**p**
Resources * Sex	5.8	1	13.5	**0.0004**
Error	0.4	82		

Table S3 continued

Wing aspect ratio	**MS**	**d.f.**	**F**	**p**
Sex	6.54	1	76.9	**<0.0001**
Family	0.19	6	2.2	0.0545
Error	0.09	76		

TABLE S4. Results of general linear mixed models for the effects of sun, sex (fixed factors), and family (random) on morphological traits in *Lycaena tityrus* for individuals released in experiment 2. Models were constructed using stepwise backwards elimination of non-significant factors. Significant p-values are given in bold.

Adult body mass	**MS**	**d.f.**	**F**	**p**
Sex	$12.2 * 10^{-4}$	1	15.4	**0.0002**
Family	$1.7 * 10^{-4}$	6	2.2	0.0525
Error	$0.8 * 10^{-4}$	80		
Thorax mass	**MS**	**d.f.**	**F**	**p**
Family	$0.2 * 10^{-4}$	6	2.4	**0.0373**
Error	$0.1 * 10^{-4}$	81		
Abdomen mass	**MS**	**d.f.**	**F**	**p**
Sun	$1.6 * 10^{-4}$	1	4.1	**0.0452**
Sex	$9.7 * 10^{-4}$	1	25.3	**<0.0001**
Sun * Sex	$1.7 * 10^{-4}$	1	4.5	**0.0365**
Family	$0.7 * 10^{-4}$	6	1.9	0.1000
Error	$0.4 * 10^{-4}$	78		

Table S4 continued

Thorax-abdomen ratio	**MS**	**d.f.**	**F**	**p**
Sun	3.26	1	20.3	**<0.0001**
Sex	12.93	1	80.6	**<0.0001**
Sun * Sex	3.44	1	21.4	**<0.0001**
Family	0.23	6	1.5	0.2021
Error	0.16	78		
Fat (mg)	**MS**	**d.f.**	**F**	**p**
Sun	2.17	1	4.2	**0.0445**
Sex	2.91	1	5.6	**0.0203**
Error	0.52	85		
Fat (%)	**MS**	**d.f.**	**F**	**p**
Sun	1509	1	22.7	**<0.0001**
Sun * Sex	647	1	9.7	**0.0025**
Error	67	85		
Wing length	**MS**	**d.f.**	**F**	**p**
Family	1.28	6	3.5	**0.0042**
Error	0.37	76		
Wing area	**MS**	**d.f.**	**F**	**p**
Sex	735.1	1	13.3	**0.0005**
Family	249.6	6	4.5	**0.0006**
Error	55.4	75		

Table S4 continued

Wing loading	**MS**	**d.f.**	**F**	**p**
Sun	1.54	1	2.3	0.1324
Sex	6.69	1	10.1	**0.0021**
Sun * Sex	2.88	1	4.3	**0.0406**
Error	0.66	79		
Wing aspect ratio	**MS**	**d.f.**	**F**	**p**
Sex	11.15	1	185.9	**<0.0001**
Family	0.13	6	2.1	0.0635
Error	0.06	75		

TABLE S5. Results of general linear mixed models for the effects of resources, sun, sex (fixed factors), family (random) and age (covariate) on morphological traits in *Lycaena tityrus* for individuals released in experiment 3. Models were constructed using a stepwise backwards elimination of non-significant factors. Significant p-values are given in bold.

Adult body mass	**MS**	**d.f.**	**F**	**p**
Sex	$14.1 * 10^{-4}$	1	21.9	**<0.0001**
Error	$0.6 * 10^{-4}$	72		
Thorax mass	**MS**	**d.f.**	**F**	**p**
Sun * Sex	$0.25 * 10^{-4}$	1	3.0	0.0869
Error	$0.08 * 10^{-4}$	72		
Abdomen mass	**MS**	**d.f.**	**F**	**p**
Sex	$15.8 * 10^{-4}$	1	53.3	**<0.0001**
Error	$0.3 * 10^{-4}$	72		
Thorax-abdomen ratio	**MS**	**d.f.**	**F**	**p**
Sex	28.32	1	147.4	**<0.0001**
Age	0.56	1	2.9	0.0933
Error	0.19	71		
Fat (mg)	**MS**	**d.f.**	**F**	**p**
Resources	2.10	1	23.0	**<0.0001**
Sex	1.24	1	13.6	**0.0004**
Error	0.09	71		

Table S5 continued

Fat (%)	**MS**	**d.f.**	**F**	**p**
Resources	874.6	1	29.3	**<0.0001**
Sex	60.8	1	2.0	0.1582
Resources * Sun	69.2	1	2.3	0.1324
Error	29.9	70		
Wing length	**MS**	**d.f.**	**F**	**p**
Age	1.55	1	2.9	0.0938
Error	0.54	68		
Wing area	**MS**	**d.f.**	**F**	**p**
Sex	255.9	1	4.0	**0.0492**
Age	1160.6	1	18.2	**<0.0001**
Error	63.8	67		
Wing loading	**MS**	**d.f.**	**F**	**p**
Sex	6.73	1	10.3	**0.0020**
Error	0.65	68		
Wing aspect ratio	**MS**	**d.f.**	**F**	**p**
Sex	8.89	1	122.4	**<0.0001**
Family	0.31	5	4.2	**0.0023**
Error	0.07	63		

4.3 Nutritional stress reduces flight performance and exploratory behavior in a butterfly

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ORIGINAL ARTICLE

Nutritional stress reduces flight performance and exploratory behavior in a butterfly

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Abstract Anthropogenic global change, including agricultural intensification and climate change, poses a substantial challenge to many herbivores due to a reduced availability of feeding resources. The concomitant food stress is expected to detrimentally affect performance, amongst others in dispersal-related traits. Thus, while dispersal is of utmost importance to escape from deteriorating habitat conditions, such conditions may negatively feedback on the ability to do so. Therefore, we here investigate the impact of larval and adult food stress on traits related to dispersal ability, including morphology, physiology, flight performance, and exploratory behavior, in a butterfly. We show that inadequate nutrition during development and in the adult stage diminishes flight performance, despite some reallocation of somatic resources. Detrimental effects of food stress on flight performance were mainly caused by reductions in body mass and storage reserves. Similar results were found for exploratory behavior. Furthermore, exploratory behavior was found to be (moderately) repeatable at the individual level, which might indicate the existence of a personality trait. This notion is further supported by the fact that flight performance and exploratory behavior were positively correlated, potentially suggesting the existence of a dispersal syndrome. In summary, our findings may have important implications for dispersal in natural environments, as the conditions requiring dispersal the most impair flight ability and thereby likely dispersal rates.

Key words condition; dispersal syndrome; flight ability; food stress; habitat degradation; resource allocation

Introduction

Dispersal, often defined as any movements potentially leading to gene flow (Ronce, 2007), defines the potential of individuals to spread, for instance away from local unfavorable conditions, to avoid competition or to more suitable habitats (Matthysen, 2012). Its incidence is predicted to increase with deteriorating environmental conditions (Bowler & Benton, 2005; Legrand *et al.*, 2015). Consequently, organisms are expected to be able to base their dispersal decisions on environmental circumstances (Dall *et al.*, 2005; Clobert *et al.*, 2009). Dispersal is therefore a crucial process to deal with heterogeneous environments (Cote & Clobert, 2007; Travis *et al.*, 2013). In the long term, it may allow individuals to track their shifting climate niche (Warren *et al.*, 2001; Hickling *et al.*, 2006) and to maintain metapopulation connectivity (Hanski & Gilpin, 1997; Baguette *et al.*, 2013).

Though dispersal is consequently an ecologically important process, the motivation to disperse and dispersal ability may differ strongly among and within populations (van Dyck & Baguette, 2005; Baguette & van Dyck, 2007; Ducatez *et al.*, 2014; Bestion *et al.*, 2015). Increasing evidence suggests that dispersing individuals are not a random subset of a given population, but that they may differ in various phenotypic traits from conspecifics, indicating the existence of so called dispersal syndromes (Bonte & Saastamoinen, 2012; Stevens *et al.*, 2013). Factors potentially affecting dispersal ability include

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morphology, physiology, and overall condition (Bowler & Benton, 2005; Bonte *et al.*, 2012; Therry *et al.*, 2014). In most insects and many other animals, dispersal is related to flight ability. Traits that have been associated with flight, and thereby at least potentially dispersal ability in flying insects, include wing size, wing aspect ratio, body mass, wing loading, thorax mass, thorax–abdomen ratio, storage reserves and flight metabolic rate (Betts & Wootton, 1988; Berwaerts *et al.*, 2002; Niitepõld *et al.*, 2009). Many of these traits show environmentally induced plasticity, which may in turn feedback on dispersal ability. Thus, if environmental conditions change, feedback loops are expected between flight-related morphology/physiology and dispersal ability.

Due to widespread effects on body size and storage reserves, food stress is certainly an important environmental factor which may detrimentally affect flight performance, as flight is a highly energy demanding process (Candy *et al.*, 1997; Suarez, 2000). In holometabolous insects, the respective energy may stem from larval storage reserves or adult income (Boggs & Freeman, 2005). Food stress in the larval phase of insect herbivores may arise from either limited availability or inadequate quality of host plants. For example, a recent study dealing with the effects of drought on plant quality revealed negative impacts of larval food stress on butterfly flight muscles (Gibbs & Weir, 2017). Larval food stress may not only diminish overall performance, but additionally trigger a reallocation of resources among competing body parts and functions such as reproduction, storage, and flight-related machinery, thus shaping the adult's physiological state (McNamara & Houston, 1996; Boggs, 2009).

Similar to larval diet, adult income also affects overall performance, as it is often of crucial importance to fuel flight, egg production, and longevity (Hill & Pierce, 1989; Fischer & Fiedler, 2001a; Bauerfeind & Fischer, 2005, 2009). Insect flight is initially fueled by carbohydrates, while longer flights rely on the lipid metabolism (Canavoso *et al.*, 2003). Consequently, inadequate nutrition during larval development and in the adult stage may exhibit negative effects on flight-related morphology and physiology. Investigating such effects seems important within the current era of anthropogenic climate change. Additionally, modern agricultural land-use practices, including fertilization, pesticide use and high mowing frequencies, will strongly reduce the availability of nectar resources for adult feeding, but also of host plants for larval feeding (Pleasants & Oberhauser, 2013; Lebeau *et al.*, 2016).

To some extent though, organisms are able to handle short-term limitations in resource availability for instance through compensatory growth (Metcalfe & Monaghan, 2001). Such compensation typically involves costs that are often apparent later in life only (Metcalfe & Monaghan, 2001; Stoks *et al.*, 2006; Block & Stoks, 2016). For instance, Saastamoinen *et al.* (2013) could demonstrate delayed costs of compensatory growth, as food-deprived individuals showed either reduced fecundity or lifespan. Likewise, shortage of larval-derived nutrients (i.e., nitrogenous compounds) limited reproduction in the tropical butterfly *Bicyclus anynana* (Bauerfeind & Fischer, 2005). Thus, the conditions experienced during development may affect the adult phenotype as a predictive adaptive response in order to be better prepared for dealing with detrimental conditions (e.g., Monaghan, 2008).

Against this background, we here investigate the effects of food stress on flight-related parameters in the nonpest butterfly *Lycaena tityrus*, a currently northward expanding species. As dispersal is expected to be a multicausal process (Matthysen, 2012; Legrand *et al.*, 2015), we target an array of different traits. As quantifying dispersal is challenging (Saastamoinen *et al.*, 2010), we here focus on proxies of dispersal ability such as morphology, physiology, and physiological flight performance (e.g., Zera *et al.*, 1998; Berwaerts *et al.*, 2002, 2006; Ducatez *et al.*, 2013; Kehl *et al.*, 2015). Additionally, we investigate exploratory behavior, a trait that may reflect different personalities in butterflies (Ducatez *et al.*, 2012, 2014). We set out to answer the following questions: (i) Does larval food stress negatively affect adult condition, flight performance, and exploratory behavior or are the butterflies able to compensate for a period of food deprivation during development? (ii) To what extend does adult starvation influence condition and flight performance? We predict that larval as well as adult food stress will negatively affect dispersal-related traits.

Materials and methods

Study organism

Lycaena tityrus (Poda, 1761) is a widespread temperate-zone butterfly ranging from Western Europe to central Asia (Ebert & Rennwald, 1991). This species is currently expanding its range toward higher latitudes and altitudes, which is assumed to be largely driven by anthropogenic climate change (Brunzel *et al.*, 2008; Settele *et al.*, 2008). The species is bivoltine with two discrete generations per year in most parts of its range. Overwintering takes place as half-grown larva. The principal larval host plant is *Rumex acetosa* L. (Polygonaceae), but several congeneric *Rumex* species are utilized as well (Ebert & Rennwald, 1991; Tolman & Lewington, 1998; Settele *et al.*, 2008).

Adults are nectar feeders, using a wide array of flowers (Ebert & Rennwald, 1991). For the following experiments, a total of 34 freshly eclosed, mated females from a bivoltine German population (vicinity of Greifswald, northeastern Germany; 54°02N, 13°26E) were caught in August 2015. The size of the respective habitat is ca. 20 ha, harboring a large population of *L. tityrus*. All females were transferred to a climate chamber at Greifswald University for egg laying.

Experimental design

Caught females were kept at 26 °C, 60% relative humidity, and a L 18 : D 6 photoperiod. Unless otherwise stated, resulting offspring was reared under the same conditions. For oviposition, females were placed separately in translucent 1 L plastic pots covered with gauze and were provided with *Rumex acetosa* (oviposition substrate), fresh flowers (e.g., *Achillea millefolium* L.), water and a highly concentrated sucrose solution (20%, v/v) for adult feeding. Deposited eggs were collected daily and transferred, separated by female, to small plastic boxes. Resulting larvae were reared under the same conditions used for oviposition. Two separate experiments were performed to investigate the effects of larval and adult food stress on the flight performance of the resulting butterflies.

Experiment 1: Larval food stress

After hatching, the larvae of each female were randomly divided among two feeding treatments (split brood design). One group had access to food in ample supply until pupation, whereas the other group experienced a period of food deprivation (30 h) in the middle of the last larval instar. This time period was used to mimic a situation in which a larva has to leave a deteriorated host plant and search for a new one. All larvae were individually reared in small plastic boxes (125 mL). Boxes contained moistened filter paper and fresh cuttings of *R. acetosa* and *acetosella*. Boxes were checked daily and larvae were supplied with fresh food as necessary. For the starvation period, food was removed from the respective boxes. Afterward, larvae were allowed to feed again until pupation. Resulting pupae were transferred to 15 °C in order to retard further development for logistic reasons, and were kept individually in plastic cups (125 mL). Once all larvae had pupated, they were back-transferred to the warmer conditions outlined above until adult eclosion. Butterflies were kept individually and were provided with moistened cotton wool for drinking. One-day-old butterflies were first subjected to a tunnel test and afterward to a flight performance test (see below). Thus, each individual was used for both flight tests. Flight performance tests were performed at 18, 22, and 26 ± 0.5 °C mimicking cold, medium, and warm summer conditions in Greifswald, while tunnel tests were performed at warm (26 ± 0.5 °C) conditions only because lower conditions likely prevent butterflies from flying. We included relatively low temperatures to test for flight performance under suboptimal conditions, which seems to be closely related to general flight performance in butterflies, and because too beneficial conditions may mask differences among treatments (Merckx *et al.*, 2006; Karl *et al.*, 2008).

Experiment 2: Adult food stress

Larvae were raised in translucent plastic boxes (125 mL) in groups of 8–10 individuals until pupation, being provided with food (*R. acetosa*) in ample supply throughout. Pupae were individually transferred to 15 °C in order to retard further development for logistic reasons. Once all larvae had pupated, pupae were back-transferred to 26 °C to induce adult eclosion. After eclosion, butterflies were randomly divided into two feeding treatments and placed individually in translucent 1 L plastic pots covered with gauze. The individuals of the control group had access to food throughout, being provided with fresh flowers, water, and a highly concentrated sucrose solution. The butterflies of the adult food stress treatment experienced a period of food deprivation for 2 d (i.e., the entire period from eclosion to testing), during which they were provided with water only. On day 2 after adult eclosion, butterflies were tested individually for flight performance at either 18 °C or 26 °C, as no significant difference was found in flight endurance between 22 and 26 °C in experiment 1 (see below). As above, butterflies were first subjected to the tunnel test and afterward to the flight performance test. Tunnel tests were also performed at 18 °C and 26 °C to test the above assumption.

Tunnel test

We used the tunnel test as previously described by Ducatez *et al.* (2012, 2014). The aim of this test is to investigate flight behavior/willingness to fly under novel and challenging conditions, thereby testing for exploratory behavior (individual boldness or shyness). To this end, we used a 3 m long opaque PVC-U pipe with a diameter of 80 cm in experiment 1. The pipe was placed on a table within a climate chamber set at a constant temperature of 26 ± 0.5 °C. In experiment 2, we used a slightly modified approach with a thinner pipe (diameter 40 cm) to increase

difficulty for the butterflies. In the climate chamber, all lights were turned off, except for a small daylight emitting light source (Beurer TL 40) at the end of the tunnel. Butterflies were individually released from handheld plastic cups in the center of the entrance of the pipe, that is, at a height of 40 cm (experiment 1) or 20 cm (experiment 2), to avoid any take-off effort. Butterflies were not touched directly to minimize handling effects. Thus, the values measured are mainly based on the behavioral decision to fly or not. Each individual was tested three times to assess behavioral repeatability (i.e., whether behavior is different between individuals but consistent across time; Bell *et al.*, 2009). For each trial, we recorded how far the butterfly flew into the tunnel toward the light source. Trials were terminated once an individual did not move for >5 s. Butterflies that flew through the entire tunnel were assigned the tunnel length of 3 m. The mean of the three trials was used in subsequent analyses.

Flight performance test

Butterflies were tested for flight performance using a well-established assay (cf. Ducatez *et al.*, 2012, 2013). Each individual was placed into a plastic chamber (30 cm × 16 cm × 14 cm), which was perforated at its base and fixed to a rapid agitator (IKA Vortex 4 digital). After a habituation period of 30 s, the vortex was switched on to strongly shake the chamber, preventing the butterfly from holding on to the walls. This method forces the butterflies to fly during the test, as they were otherwise lying uncomfortably on the bottom of the shaking chamber. The time an individual butterfly spent flying was recorded for 60 s, with higher values reflecting a better flight performance. After the performance test, butterflies were frozen for later analyses of potentially flight-related parameters.

Morphological and physiological analyses

To test for associations between flight behavior/performance and other traits, we measured several morphological and physiological traits. First, adult body mass was determined to the nearest 0.01 mg (Sartorius CPA225D). Then, wings, heads, and legs were removed. Thorax and abdomen were separated and afterward weighed. Abdomen fat content was measured following Fischer *et al.* (2003), but using the less poisonous acetone instead of dichloromethane. Abdomens were first dried to constant weight for 2 d at 70 °C. Abdomen dry mass was measured. Afterward, fat was extracted for two times 48 h using 1 mL of acetone for each butterfly. Solutions were exchanged between both fat extractions. Then, abdomens were again dried for 2 d at 70 °C, after which fat-free dry mass was measured. Absolute (mg) and relative (%) abdomen fat content were determined as the mass difference between abdomen dry mass and the remaining dry mass after the two fat extractions. Forewing area and length (from basis to apex) were measured using digital images of left forewings (captured from ventrally with a digital camera mounted on a stereo microscope) and NIS elements software. Wing loading was calculated as total body mass divided by forewing area, and wing aspect ratio as 4 × forewing length^2 divided by the forewing area (Berwaerts *et al.*, 2002). Thorax–abdomen ratio was calculated by dividing thorax through abdomen mass.

Statistical analyses

Data on developmental, morphological and physiological traits were analyzed with general linear mixed models (GLMMs; experiment 1) and general linear models (GLMs; experiment 2), using feeding treatment and sex as well as the respective interaction as fixed factors. In experiment 1, family (i.e., the offspring of each individual female) was used additionally as a random effect. Only families with $n > 7$ offspring were considered to cover within-family variation. In experiment 2 consideration of family was not possible as according data were not available. In experiment 1, data on abdomen mass and in experiment 2 data on abdomen mass, thorax–abdomen ratio, and absolute fat content were LN transformed prior to analysis to meet model requirements. Data on exploratory behavior and flight performance were also analyzed with GLMs, using the same factors mentioned above and additionally test temperature for flight endurance in the vortex and the tunnel test in experiment 2. For experiment 1, data on the flight distance in the tunnel were LN transformed and on flight endurance x^3 transformed prior to analyses. Interaction terms between feeding treatment, sex, and test temperature (if used) were also included.

To additionally test for the impact of continuous variables (morphology, physiology) on exploratory behavior and flight performance, we first performed principal component analyses (PCAs) owing to strong intercorrelations among traits. PCAs were calculated separately for males and females, as sexes differed strongly in a variety of traits (see below). In experiment 1, we used the first five principal components (PCs) for further analyses, having Eigenvalues between 3.1 and 0.9 for males and between 3.7 and 1.0 for females (cf. Table 1). In experiment 2, we used the first four PCs for further analyses, having Eigenvalues between 3.9 and 1.0 for males and between 5.7 and 0.8 for females (cf. Table 2). As PCAs had to be

Table 1 Results of a principal component analyses for larval starved males (A) and females (B) including Eigen values, percentage of the variance explained, cumulative percentage of the variance explained, and *r* values of correlations with continuous variables (rows 4–13) for principal components (PC) 1–5 (experiment 1). *r* values > 0.7 in bold.

No.	Parameter	PC1	PC2	PC3	PC4	PC5
(A) Males						
1	Eigen value	3.1	2.4	1.9	1.7	0.9
2	Variance explained (%)	30.8	23.8	19.2	16.6	8.5
3	Cumulative variance (%)	30.8	54.6	73.8	90.4	98.9
4	Adult body mass (mg)	**0.956**	0.261	−0.061	0.038	0.017
5	Thorax mass (mg)	**0.862**	0.248	−0.111	−0.379	0.093
6	Abdomen mass (mg)	**0.723**	0.121	0.102	0.650	−0.139
7	Thorax–abdomen ratio	0.095	0.157	−0.187	**−0.930**	0.234
8	Fat (mg)	0.297	−0.318	**0.880**	−0.098	0.112
9	Fat (%)	0.070	−0.352	**0.903**	−0.147	0.141
10	Wing length (mm)	0.009	**0.784**	0.346	−0.222	−0.464
11	Wing area (mm^2)	−0.048	**0.951**	0.230	0.029	0.003
12	Wing loading (mg/cm^2)	**0.884**	−0.386	−0.241	−0.004	0.032
13	Wing aspect ratio	0.118	−0.568	−0.015	−0.376	**−0.722**
(B) Females						
1	Eigen value	3.7	2.3	1.7	1.3	1.0
2	Variance explained (%)	37.0	22.6	17.0	13.0	9.6
3	Cumulative variance (%)	37.0	59.7	76.6	89.5	99.1
4	Adult body mass (mg)	**0.963**	0.174	−0.188	−0.016	0.042
5	Thorax mass (mg)	**0.850**	0.234	−0.361	0.194	0.216
6	Abdomen mass (mg)	**0.925**	0.078	0.078	−0.293	−0.197
7	Thorax–abdomen ratio	−0.265	0.173	−0.503	0.630	0.497
8	Fat (mg)	0.618	−0.339	0.620	0.275	0.113
9	Fat (%)	0.206	−0.449	**0.702**	0.458	0.174
10	Wing length (mm)	0.139	**0.855**	0.206	0.343	−0.299
11	Wing area (mm^2)	0.133	**0.928**	0.336	0.024	0.074
12	Wing loading (mg/cm^2)	**0.817**	−0.410	−0.396	−0.023	0.008
13	Wing aspect ratio	−0.013	−0.256	−0.285	0.606	−0.697

constructed separately for males and females, PCs could not be included in the above models testing for sexual differences in flight performance. We therefore constructed another set of models separately for each sex, by including the respective PCs and the results of tunnel or vortex test as covariates, followed by a stepwise backward elimination of nonsignificant factors. All statistical tests were performed with Statistica 12.0 (Tulsa, StatSoft, OK, USA). All means are given ±1 SE and are based on untransformed data for easier reference.

Results

Experiment 1: Larval food stress

Developmental and morphological traits were strongly affected by feeding treatment and sex (Table 3). Feeding treatment significantly affected larval development time, adult body mass, abdomen mass, thorax mass, wing length, wing area, and wing loading, but not pupal development time, thorax–abdomen ratio, abdomen fat content, and wing aspect ratio. Food stress resulted in longer larval development times, lower body masses, smaller wings, and a lower wing loading (for mean values see Table S1). Feeding treatment by sex interactions were significant for larval and pupal development time only, showing that females responded more strongly to food stress than males. Significant sex differences were found for all traits except from thorax mass, wing length, and wing area, indicating that females had on average longer development times, higher abdomen and concomitantly total body masses, a lower thorax–abdomen ratio, higher absolute but lower relative abdomen fat contents, a higher wing loading, and a lower wing aspect ratio than males. Additionally,

Table 2 Results of a principal component analyses for adult starved males (A) and females (B) including Eigen values, percentage of the variance explained, cumulative percentage of the variance explained, and *r* values of correlations with continuous variables (rows 4–13) for principal components (PC) 1–4 (experiment 2). *r* values > 0.7 in bold.

No.	Parameter	PC1	PC2	PC3	PC4
(A) Males					
1	Eigen value	3.9	2.9	1.3	1.0
2	Variance explained (%)	39.4	29.0	13.2	10.1
3	Cumulative variance (%)	39.4	68.4	81.6	91.7
4	Adult body mass (mg)	**0.953**	−0.084	0.256	−0.060
5	Thorax mass (mg)	0.588	−0.670	0.002	−0.287
6	Abdomen mass (mg)	**0.820**	0.420	0.297	−0.001
7	Thorax–abdomen ratio	−0.299	**−0.771**	−0.308	−0.284
8	Fat (mg)	0.655	0.323	−0.636	0.105
9	Fat (%)	0.580	0.187	**−0.737**	0.199
10	Wing length (mm)	0.425	**−0.831**	0.053	0.234
11	Wing area (mm^2)	0.509	**−0.759**	−0.006	−0.076
12	Wing loading (mg/cm^2)	**0.840**	0.336	0.309	−0.027
13	Wing aspect ratio	−0.094	0.397	0.159	**0.857**
(B) Females					
1	Eigen value	5.7	2.0	1.0	0.8
2	Variance explained (%)	57.1	20.1	10.0	7.7
3	Cumulative variance (%)	57.1	77.3	87.3	95.0
4	Adult body mass (mg)	**0.981**	−0.050	−0.040	−0.105
5	Thorax mass (mg)	**0.804**	−0.404	−0.119	0.242
6	Abdomen mass (mg)	**0.964**	0.088	−0.090	−0.202
7	Thorax–abdomen ratio	**−0.785**	−0.387	0.040	0.427
8	Fat (mg)	**0.770**	0.413	0.279	0.228
9	Fat (%)	0.432	0.586	0.468	0.453
10	Wing length (mm)	0.584	**−0.763**	0.204	0.069
11	Wing area (mm^2)	**0.725**	−0.606	−0.123	0.214
12	Wing loading (mg/cm^2)	**0.920**	0.205	0.014	−0.237
13	Wing aspect ratio	−0.262	−0.427	**0.790**	−0.343

females tended to have larger wings than males. Based on the above data we performed two PCAs, one for each sex. In both sexes the resulting first five PCs were most strongly related to body mass (PC1), wing size (PC2), abdomen fat content (PC3), thorax–abdomen ratio (PC4), and wing aspect ratio (PC5; Table 1).

Flight performance traits were first analyzed with GLMMs including the factors feeding treatment, sex, and family. In the tunnel test, exploratory behavior was significantly affected by the factors feeding treatment, sex, and family (Table 4, part A). Control individuals and males covered longer distances than food-stressed individuals and females, respectively (Fig. 1A). In a next step, we calculated GLMMs separately for males and females to include PCs as covariates. For males, including covariates revealed significant effects of feeding treatment, PC3 (reflecting fat content), and the results of the vortex test, while family was not included anymore. Flight distance was positively related to PC3 (beta 0.17 ± 0.09), suggesting that flight distance tended to be positively related to abdomen fat content in males. Furthermore, flight distance was positively correlated with flight performance (beta 0.17 ± 0.08). Hence, males that covered longer distances in the tunnel also showed a better flight performance in the vortex. For females, in contrast, no parameters were selected. Overall, the repeatability of the tunnel test was moderate but significant, with correlations among the three replicated measures being positive with *r* values ranging between 0.464 and 0.568 (all $P < 0.0001$, $n = 301$).

In the vortex test, flight performance was significantly affected by test temperature and feeding treatment. Flight

Table 3 General linear mixed model results for the effects of larval feeding treatment, sex (both fixed), and family (random factor) on developmental and morphological traits in *Lycaena tityrus* (experiment 1). When not attaining significance, interactions and the random effect "family" were removed in a stepwise manner. Significant *P* values are given in bold.

Traits	MS	DF	DF error	*F*	*P*
Larval time (d)					
Feeding treatment	43.0	1	277	17.5	**<0.0001**
Sex	438.5	1	277	178.3	**<0.0001**
Feeding × sex	16.7	1	277	6.8	**0.0097**
Pupal time (d)					
Feeding treatment	15.7	1	277	2.5	0.1166
Sex	184.0	1	277	29.1	**<0.0001**
Feeding × sex	122.0	1	277	19.3	**<0.0001**
Adult body mass (mg)					
Feeding treatment	1711.4	1	279	78.9	**<0.0001**
Sex	1659.2	1	279	76.5	**<0.0001**
Abdomen mass (mg)					
Feeding treatment	1.8	1	279	34.0	**<0.0001**
Sex	14.4	1	279	279.8	**<0.0001**
Thorax mass (mg)					
Feeding treatment	514.5	1	279	77.1	**<0.0001**
Sex	15.7	1	279	2.4	0.1257
Thorax–abdomen ratio					
Feeding treatment	0.5	1	279	3.5	0.0613
Sex	37.9	1	279	283.9	**<0.0001**
Fat (absolute)					
Feeding treatment	0.01	1	279	1.0	0.3218
Sex	0.53	1	279	59.3	**<0.0001**
Fat (relative)					
Feeding treatment	39.2	1	279	3.1	0.0800
Sex	342.8	1	279	27.0	**<0.0001**
Wing length (mm)					
Feeding treatment	35.8	1	279	77.4	**<0.0001**
Sex	0.4	1	279	0.9	0.3432
Wing area (mm^2)					
Feeding treatment	5363	1	279	69.8	**<0.0001**
Sex	280	1	279	3.7	0.0571
Wing loading (mg/cm^2)					
Feeding treatment	279	1	279	5.2	**0.0228**
Sex	1795	1	279	33.8	**<0.0001**
Wing aspect ratio					
Feeding treatment	0.4	1	279	1.1	0.3057
Sex	1.8	1	279	4.6	**0.0334**

Table 4 General linear model results for (A) the effects of larval feeding treatment, sex, and family on the flight distance covered in a tunnel and (B) for the effects of larval feeding treatment, test temperature, sex, and family on flight endurance in a vortex in *Lycaena tityrus* (experiment 1). Second models were constructed by additionally including PCs 1–5 and the flight performance in the tunnel or in the vortex as covariates, followed by a stepwise backward elimination of nonsignificant factors. The latter models were constructed separately for males and females, owing to strong variation in morphological traits among the sexes (cf. Table S1). Throughout, family was included as random effect, all others as fixed effects. For (A) no factor was selected in model 2 for females. Significant *P* values are given in bold.

Models	MS	DF	DF error	*F*	*P*
(A)					
Tunnel test model 1					
Feeding treatment	5.0	1	277.5	8.5	**0.0039**
Sex	3.7	1	277.4	6.4	**0.0121**
Family	1.1	22	256.0	2.0	**0.0061**
Males model 2					
Feeding treatment	2.9	1	141	4.5	**0.0351**
PC3 (fat content)	2.6	1	141	4.1	**0.0439**
Vortex test	3.0	1	141	4.7	**0.0320**
Females model 2					
No factors selected					
(B)					
Vortex test model 1					
Feeding treatment	1.5×10^{10}	1	275	4.1	**0.0429**
Temperature	8.2×10^{10}	1	275	22.5	**<0.0001**
Sex	1.2×10^{10}	1	275	3.3	0.0708
Males model 2					
Temperature	5.7×10^{10}	2	140	16.7	**<0.0001**
PC1 (body mass)	1.7×10^{10}	1	140	5.1	**0.0256**
Tunnel test	1.7×10^{10}	1	140	5.0	**0.0272**
Females model 2					
Temperature	4.0×10^{10}	2	130	11.7	**<0.0001**
PC1 (body mass)	1.6×10^{10}	1	130	4.6	**0.0339**
Tunnel test	1.2×10^{10}	1	130	3.5	0.0646

endurance increased with increasing test temperature and was higher in control than in starved individuals (Table 4, part B, Fig. 1B). Additionally, females tended to show a shorter flight endurance than males. Including covariates in the subsequent analyses separated by sex revealed significant effects of temperature and PC1 (reflecting body mass) in both sexes, and the performance in the vortex test in males, while feeding treatment was not included anymore. The latter indicates that the effects of feeding treatment are mediated by variation in PC1, that is, body mass. Flight endurance was positively related to PC1 (males: beta 0.17 ± 0.08; females: beta 0.20 ± 0.08). Thus, high body mass enhanced flight endurance. Flight performance was positively correlated with the tunnel test results in males (beta 0.17 ± 0.08). Hence, males that covered longer distances in the tunnel also showed a better flight performance. In females, flight distance in the tunnel test tended to be positively correlated to flight performance (beta 0.15 ± 0.09). In general, results of the tunnel test and vortex test were positively related (Spearman's correlation: $r = 0.19$, $P < 0.05$; Fig. S1A).

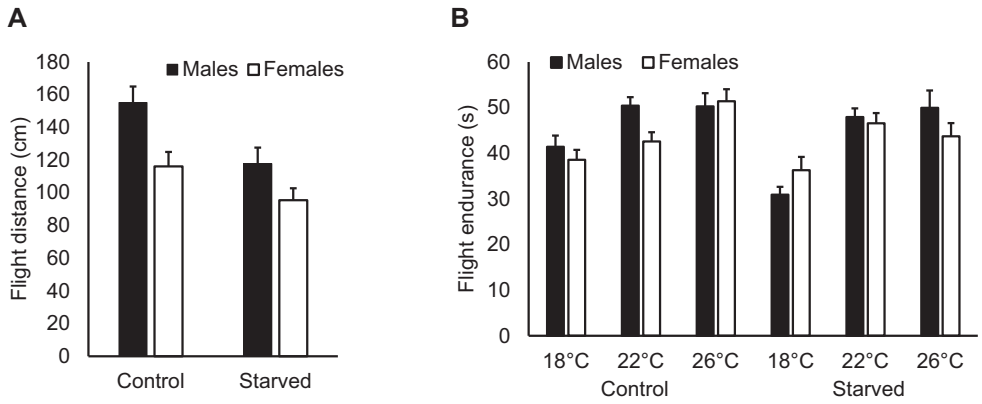

Fig. 1 Flight distance covered in a tunnel (A) and flight endurance in a vortex (B, means + 1 SE) in *Lycaena tityrus* males and females in relation to larval feeding treatment and test temperature (in B, experiment 1). Group sample sizes range between 74 and 77 (in A) and 24 and 26 (in B). Males: filled bars; females: open bars.

Experiment 2: Adult food stress

Regarding morphological and physiological traits, adult feeding treatment significantly affected adult body mass, abdomen mass, thorax mass, thorax–abdomen ratio, absolute and relative fat content, wing loading, and wing aspect ratio, but not wing length and wing area (Table 5). Absence of adult food sources resulted in lower abdomen, thorax, and body masses, a higher thorax–abdomen ratio and reduced wing loading, lower absolute and relative fat contents, and a higher wing aspect ratio (for means see Table S2). Significant sex differences were found for all traits measured, except for wing length. Body and abdomen mass, absolute fat content, wing area, and wing loading were higher while thorax mass, thorax–abdomen ratio and wing aspect ratio were lower in females than in males. Females tended to have smaller wings than males. Sexual differences in adult body mass and concomitantly wing loading though were restricted to control individuals (significant feeding treatment by sex interactions). Again, PCAs were performed for both sexes separately. In males, the extracted PCs represent in the first place body mass (PC1), wing size and thorax–abdomen ratio (PC2), fat content (PC3), and wing aspect ratio (PC4). In females, the PCs reflect mainly body mass (PC1), wing size (PC2), wing aspect ratio (PC3), and fat content and thorax–abdomen ratio (PC4; cf. Table 2).

In the GLM including the factor sex, the distance covered in the tunnel was significantly affected by the factor temperature only (Table 6, part A), with butterflies covering longer distances at the higher temperature (26 °C: 30.7 ± 3.3 cm, 18 °C: 2.3 ± 6.3 cm; Fig. 2A). Including subsequently principal components and the flight performance in the vortex as covariates did not reveal additional significant effects. Overall, the repeatability of the tunnel test was weak but significant in two out of three cases, with r values ranging between 0.142 and 0.295 (trial 1 and 2: $P = 0.0045$; trial 1 and 3: $P = 0.0001$; trial 2 and 3: $P = 0.0685$; $n = 165$).

In the vortex test, flight performance was significantly affected by feeding treatment and temperature, with flight endurance being higher at the higher temperature and in control than in starved individuals (Table 6, part B; Fig. 2B). Including principal components and the results of the tunnel test as covariates revealed significant effects of feeding treatment, PC1 (reflecting body mass), and the tunnel test results for males, and of temperature for females. Additionally, PC4 (reflecting relative fat content and thorax–abdomen ratio) tended to affect flight performance in females. In males, flight performance was positively correlated with PC1 (beta 0.28 ± 0.12) and the tunnel test results (beta 0.32 ± 0.11). Thus, high body mass increased flight performance and males that covered longer distances in the tunnel also had a better flight performance. In females, flight performance tended to be positively correlated with PC4 (beta 0.17 ± 0.09). Thus, a high relative fat content and thorax–abdomen ratio tended to increase flight performance. In general, results of the tunnel test and vortex test were positively related (Spearman's correlation: $r = 0.22$, $P < 0.05$; Fig. S1B).

Discussion

Effects of feeding treatment on morphological and physiological traits

Food stress during larval development resulted, as expected, in longer development time, lower body mass and

Table 5 General linear model results for the effects of adult feeding treatment and sex (both fixed) on morphological and physiological traits in *Lycaena tityrus* (experiment 2). Non-significant interactions were removed from models in a stepwise manner. Significant *P* values are given in bold.

Traits	MS	DF	*F*	*P*
Adult body mass (mg)				
Feeding treatment	12.3×10^{-4}	1	26.0	**<0.0001**
Sex	5.6×10^{-4}	1	11.8	**0.0008**
Feeding × sex	3.6×10^{-4}	1	7.7	**0.0061**
Error	0.5×10^{-4}	162		
Abdomen mass (mg)				
Feeding treatment	5.3	1	53.9	**<0.0001**
Sex	8.7	1	88.1	**<0.0001**
Error	0.1	163		
Thorax mass (mg)				
Feeding treatment	0.33×10^{-4}	1	4.0	**0.0485**
Sex	0.68×10^{-4}	1	8.2	**0.0048**
Error	0.08×10^{-4}	163		
Thorax–abdomen ratio				
Feeding treatment	3.3	1	31.8	**<0.0001**
Sex	10.8	1	105.7	**<0.0001**
Error	0.1	163		
Fat (absolute)				
Feeding treatment	30.4	1	44.4	**<0.0001**
Sex	13.2	1	19.3	**<0.0001**
Error	0.7	163		
Fat (relative)				
Feeding treatment	475.8	1	18.8	**<0.0001**
Sex	116.1	1	4.6	**0.0339**
Error	25.4	163		
Wing length (mm)				
Feeding treatment	0.3	1	0.6	0.4371
Sex	1.8	1	3.6	**0.0582**
Error	0.5	161		
Wing area (mm^2)				
Feeding treatment	25	1	0.4	0.5084
Sex	464	1	8.1	**0.0050**
Error	57	161		
Wing loading (mg/cm^2)				
Feeding treatment	1429	1	41.8	**<0.0001**
Sex	295	1	8.6	**0.0038**
Feeding × sex	228	1	6.7	**0.0107**
Error	34	160		
Aspect ratio				
Feeding treatment	1.6	1	12.6	**0.0005**
Sex	18.8	1	148.2	**<0.0001**
Error	0.1	161		

Table 6 General linear model results for the effects of adult feeding treatment, temperature, and sex (all fixed) on the flight distance covered in a tunnel (A) and on flight endurance in a vortex (B) in *Lycaena tityrus* (experiment 2). Second models were constructed by additionally including PCs 1–4 and the flight performance in tunnel or vortex as covariates, followed by a stepwise backward elimination of nonsignificant factors. The latter models were constructed separately for males and females, owing to strong variation in morphological traits among the sexes (cf. Table S2). For (A), no PCs were included in either males or females. Significant *P* values are given in bold.

Models	MS	DF	*F*	*P*
(A)				
Tunnel test model 1				
Temperature	28699.8	1	23.96	**<0.0001**
Error	1198.0	164		
Males model 2				
No factors selected				
Females model 2				
No factors selected				
(B)				
Vortex test model 1				
Feeding treatment	2192.1	1	7.33	**0.0075**
Temperature	9711.6	1	32.46	**<0.0001**
Error	299.2	163		
Males model 2				
Feeding treatment	1228.4	1	4.2	**0.0445**
PC1 (body mass)	1709.9	1	5.9	**0.0185**
Tunnel test	2394.0	1	8.2	**0.0058**
Error	291.3	58		
Females model 2				
Temperature	7766.3	1	27.1	**<0.0001**
PC4 (aspect ratio)	1079.1	1	3.8	0.0551
Error	286.5	99		

wing loading, and an overall smaller adult size. Thus, despite a prolonged larval period, individuals remained smaller (accompanied by lower wing loadings) compared with individuals that developed under control conditions (Blanckenhorn, 1999; Fischer & Fiedler, 2001b; Boggs & Freeman, 2005; Bauerfeind & Fischer, 2009). This could suggest that development time and body size are both critically important to fitness. Otherwise larvae should further increase larval time to avoid size reductions (in case of a selective premium on body size) or not extend the larval time at all (in case of a selective premium on larval time; Bauerfeind & Fischer, 2005).

Interestingly, only the females showed longer larval and pupal development times after larval food stress,

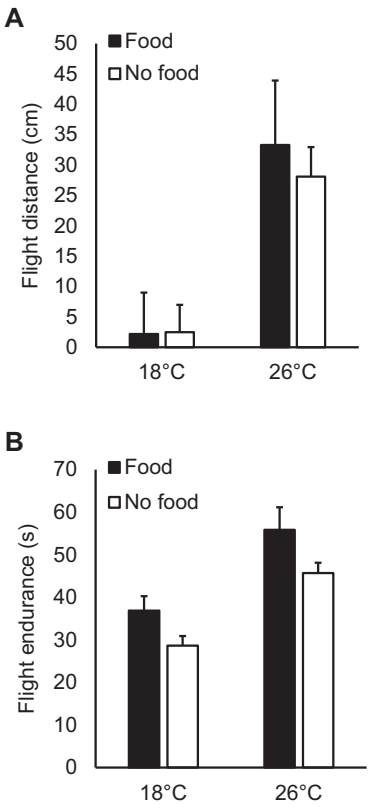

Fig. 2 Flight distance covered in a tunnel (A) and flight endurance in a vortex (B, means + 1 SE) in *Lycaena tityrus* in relation to adult feeding treatment and test temperature (experiment 2). Group sample sizes range between 26 and 59 individuals. Food: filled bars; no food: open bars.

suggesting that the above general patterns differ among the sexes. Thus, males seem to have a selective premium on fast development to ensure early emergence that is assumed to increase mating success (protandry selection; Wiklund & Fagerström, 1977; Fischer & Fiedler, 2000; Karl & Fischer, 2008), which likely explains the lack of response in development time found here (Karl *et al.*, 2010). Females, in contrast, did prolong development time, suggesting a premium on body size, which is often associated with increased fecundity (Bauerfeind & Fischer, 2005). In general, the observed sex differences with females being larger than males reflect well-known and expected patterns found in many insects, which most likely result from differential selective pressures (Gilchrist, 1990; van Dyck & Wiklund, 2002; Merckx & van Dyck, 2005; Berwaerts *et al.*, 2006). It is striking that abdomen fat content remained unaffected by larval food stress, which may aid dispersal and/or investment into reproduction as fat is the principal energy store in insects (e.g., Karl & Fischer, 2008).

Effects of adult food stress on body mass were stronger in females than in males, which is likely attributable to a lack of egg production under food deprivation. Note that Copper butterflies are strongly relying on adult feeding for egg production (Fischer & Fiedler, 2001a). Although attaining significance, the effect of food stress on thorax mass was comparatively weak, suggesting that butterflies may have tried to preserve thoracic muscles and thus flight performance (high thorax–abdomen ratio; Marden, 1989; Thomas *et al.*, 1998; Berwaerts *et al.*, 2002) to enhance the ability to disperse to a more suitable habitat (Clobert *et al.*, 2009). However, note that in insects storage reserves are mainly conserved within the abdomen, such that effects of food shortage are less likely to affect thorax as compared with abdomen mass. We have no explanation for the effect of adult food stress on wing aspect ratio, which is determined prior to adult eclosion. Taken together, the above results indicate some changes in somatic allocation between control and food-stressed individuals in order to maintain dispersal ability. Furthermore, they clearly show that our feeding treatments were successful in manipulating morphology and physiology, a necessary prerequisite to examine such variation on flight performance.

Effects on exploratory behavior and flight performance

Flight behavior was measured here using two established laboratory assays, a tunnel test and flight endurance. Interestingly, the results of both tests were statistically (though weakly) related, such that individuals with a higher flight endurance also performed better in the tunnel test. These findings suggest that either the tunnel test measures flight ability along with exploratory behavior, or that individuals in better condition (as evidenced by a better performance in the vortex) are more likely to exhibit exploratory behavior. Anyway, it is striking that in our study both approaches yielded relatively similar results.

We were able to demonstrate that starvation during development as well as in the adult stage may have detrimental consequences for flight performance and thus potentially dispersal ability. Flight endurance in the vortex was in both experiments reduced in food-stressed individuals. Larval starvation additionally negatively affected the distance covered in the tunnel. A lack of adequate nutrition has often negative impacts on adult physiology and overall performance of organisms (Boggs, 2009; Hamel *et al.*, 2009), as also indicated in our study. Interestingly, a study of Saastamoinen *et al.* (2010) revealed an opposite

pattern, namely that food-stressed individuals performed better during a flight test than control individuals, perhaps due to an altered resource allocation. In our study though flight performance was clearly reduced through food stress, which might be problematic in the current era of anthropogenic global change. While increasing habitat change and fragmentation warrant dispersal, rising temperatures and habitat degradation diminish habitat quality, which may negatively feedback on dispersal ability through increased food stress. Note that such effects may be exaggerated by (interactions with) additional stressors associated with climate change, including desiccation and heat stress. For instance, exposure of fruit flies to food and heat stress decreased flight distance and duration in comparison to flies experiencing no-stress treatments (Wang *et al.*, 2009). Likewise, an exposure to high temperatures during development decreased flight metabolic rates and hence likely flight performance in the Glanville fritillary butterfly (Mattila & Hanski, 2014).

Measuring morphological and physiological traits and including them as covariates in statistical models may shed further light on the mechanistic underpinning of such reduced performance under food stress. After including covariates, feeding treatment only remained marginally significant for larval food-stressed males in the tunnel test and adult food-stressed males in the vortex test. These findings suggest that the poorer performance of food-stressed individuals is mechanistically in the first place caused by stress-induced changes in morphology and physiology. Specifically, body mass and relative fat content seemed to be most relevant here. Body mass was positively related to flight endurance (vortex test) in males in both experiments, and in females having experienced larval food stress. Moreover, flight endurance after adult food stress tended to be positively related to relative fat content in females. These findings suggest that individuals that managed to maintain high body mass and thereby amounts of storage reserves in periods of food stress showed a better flight performance. Storage reserves such as lipids are commonly used to fuel flight activity, reflect a generally good condition, and are related to acceleration capacity (Zera *et al.*, 1998; Berwaerts *et al.*, 2002; Karl & Fischer, 2008). Similar results were obtained in the tunnel test with males that had experienced larval starvation, in which the distance covered in the tunnel was positively related to relative fat content.

Sex differences in flight performance were obvious in the larval food stress experiment only. Here, females showed a tendency to perform worse in comparison with males in both flight tests. This might be due to their higher mass and thus wing loading, which leads to higher energetic costs during flight (Wickman, 2009). This does not necessary contradict our above conclusions. While those individuals dealing with food stress best may have a generally better condition, female flight ability may nevertheless suffer from their generally higher wing loading.

Ambient temperature, the most important environmental factor for ectotherms (e.g., Deutsch *et al.*, 2008), is mainly determining body temperature (Wickman, 2009). Body temperature in turn influences the flight performance of ectotherms because physiology is temperature dependent (Huey & Kingsolver, 1993; Gilchrist, 1996; Forsman, 1999). Our results thus confirm other findings, which revealed positive relationships between high temperatures and flight performance (Forsman, 1999).

Conclusions

In our study, we were able to demonstrate that inadequate nutrition negatively affected flight performance. This is true despite some re-allocation of somatic resources in favor of dispersal-related traits. Furthermore, higher temperatures increased flight performance. This suggests that flight performance, and presumably also dispersal, are strongly dependent on environmental conditions such as ambient temperatures. Detrimental effects of starvation on flight performance seem to be conveyed in the first place by reductions in body mass and storage reserves, and thus the total amount of resources being available to the given individual. Our findings may have important implications for dispersal in natural environments. While deteriorating habitat conditions are expected to promote dispersal as an adaptive response (Legrand *et al.*, 2015), they may at the same time interfere with an individual's ability to do so.

The tunnel test used here was previously developed to detect possible behavioral differences between individuals under unfavorable conditions (Ducatez *et al.*, 2012, 2014; Ducatez & Baguette, 2016), while the vortex test addresses flight ability. Here, the latter produced more clear-cut results. Nevertheless, the tunnel test was shown to produce (moderately) repeatable results at the individual level, which might indicate the existence of personality traits in butterflies. Interestingly, both tests yielded similar results and were statistically correlated, which might point toward the existence of a dispersal syndrome (e.g., for *Pieris brassicae* in Ducatez *et al.*, 2012). Thus, the individuals performing repeatedly better than others in different types of flight tests might be the ones more apt to disperse under natural conditions (Delattre *et al.*, 2013).

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Disclosure

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Developmental and morphological traits in relation to larval feeding regime (starved individuals were deprived of food for 30 h in the last larval instar) and sex in *Lycaena tityrus* (experiment 1).

Table S2 Morphological and physiological traits in relation to adult feeding regime and sex in *Lycaena tityrus* (experiment 2).

Fig. S1 Relationship between the tunnel and vortex test for experiment 1(A) and 2(B).

Supporting Information

Table S1 Developmental and morphological traits in relation to larval feeding regime (starved individuals were deprived of food for 30 h in the last larval instar) and sex in *Lycaena tityrus* (experiment 1). Given are means ± 1 SE. Group sample sizes range between 74 and 78 individuals. Different superscript letters (a, b, c, d) within a row indicate significant differences among groups (Tukey HSD after GLM). Thus, means followed by the same letter (e.g. 'a') do not differ significantly, while means followed by different letters (e.g. 'a' and 'b') do differ significantly.

Trait	Males fed	Females fed	Males starved	Females starved
Larval time (d)	19.7 ± 0.16^{a}	21.7 ± 0.20^{b}	20.0 ± 0.22^{a}	23.0 ± 0.16^{c}
Pupal time (d)	26.3 ± 0.25^{ab}	26.6 ± 0.32^{a}	25.5 ± 0.26^{b}	28.4 ± 0.36^{c}
Adult body mass (mg)	29.6 ± 0.51^{a}	35.0 ± 0.55^{b}	25.2 ± 0.50^{c}	29.5 ± 0.66^{a}
Abdomen mass (mg)	7.6 ± 0.31^{a}	12.1 ± 0.30^{b}	6.6 ± 0.18^{c}	10.1 ± 0.30^{d}
Thorax mass (mg)	15.7 ± 0.32^{a}	16.1 ± 0.24^{a}	13.0 ± 0.31^{b}	13.4 ± 0.34^{b}
Thorax-abdomen ratio	2.2 ± 0.06^{a}	1.4 ± 0.02^{b}	2.0 ± 0.05^{a}	1.4 ± 0.03^{b}
Fat (mg)	0.1 ± 0.01^{a}	0.2 ± 0.01^{b}	0.1 ± 0.01^{a}	0.2 ± 0.01^{b}
Fat (%)	6.6 ± 0.46^{ac}	4.6 ± 0.27^{b}	7.5 ± 0.55^{c}	5.2 ± 0.33^{ab}
Wing length (mm)	14.4 ± 0.07^{a}	14.6 ± 0.06^{a}	13.8 ± 0.09^{b}	13.8 ± 0.10^{b}
Wing area (mm^2)	83.2 ± 1.06^{a}	86.1 ± 0.83^{a}	75.4 ± 1.13^{b}	76.4 ± 1.11^{b}
Wing loading	36.1 ± 0.84^{ac}	40.9 ± 0.78^{b}	33.9 ± 0.81^{c}	39.1 ± 1.04^{ab}
Wing aspect ratio	10.1 ± 0.10^{a}	10.0 ± 0.08^{a}	10.2 ± 0.06^{a}	10.0 ± 0.05^{a}

Table S2 Morphological and physiological traits in relation to adult feeding regime and sex in *Lycaena tityrus* (experiment 2). Given are means ± 1 SE. Group sample sizes range between 24 and 55 individuals. Different superscript letters (a, b, c, d) within a row indicate significant differences among groups (Tukey HSD after GLM). Thus, means followed by the same letter (e.g. 'a') do not differ significantly, while means followed by different letters (e.g. 'a' and 'b') do differ significantly.

Trait	Males fed	Females fed	Males starved	Females starved
Adult body mass (mg)	34.7 ± 1.1^{a}	41.6 ± 1.3^{b}	32.1 ± 0.8^{a}	32.8 ± 0.8^{a}
Abdomen mass (mg)	11.3 ± 0.8^{ac}	19.1 ± 0.9^{b}	8.3 ± 0.4^{a}	12.3 ± 0.5^{c}
Thorax mass (mg)	17.3 ± 0.5^{a}	16.4 ± 0.4^{ab}	17.0 ± 0.8^{a}	15.1 ± 0.3^{b}
Thorax-abdomen ratio	1.8 ± 0.11^{a}	0.9 ± 0.03^{b}	2.1 ± 0.12^{c}	1.3 ± 0.03^{d}
Fat (mg)	3.1 ± 0.04^{a}	5.7 ± 0.06^{b}	1.5 ± 0.03^{a}	2.0 ± 0.02^{a}
Fat (%)	9.8 ± 0.8^{a}	8.7 ± 1.0^{a}	7.4 ± 1.3^{ab}	4.7 ± 0.3^{b}
Wing length (mm)	14.8 ± 0.1^{a}	14.7 ± 0.1^{a}	15.1 ± 0.1^{a}	14.7 ± 0.1^{a}
Wing area (mm^{2})	83.5 ± 1.2^{a}	89.0 ± 1.1^{b}	85.7 ± 1.3^{ab}	86.5 ± 1.0^{ab}
Wing loading	41.2 ± 1.0^{a}	46.5 ± 1.0^{b}	37.5 ± 0.6^{ac}	37.8 ± 0.7^{c}
Wing aspect ratio	10.5 ± 0.1^{a}	9.7 ± 0.1^{b}	10.6 ± 0.1^{a}	10.0 ± 0.1^{c}

Figure S1 Relationship between the tunnel and vortex test for experiment 1 (A) and 2 (B).

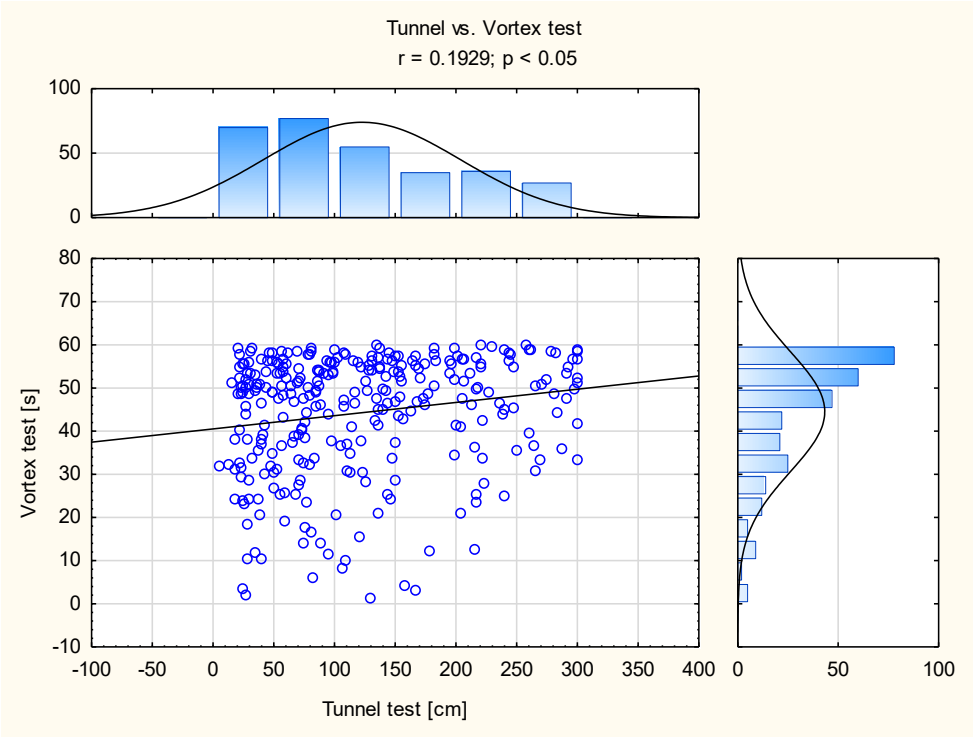

Fig. S1A

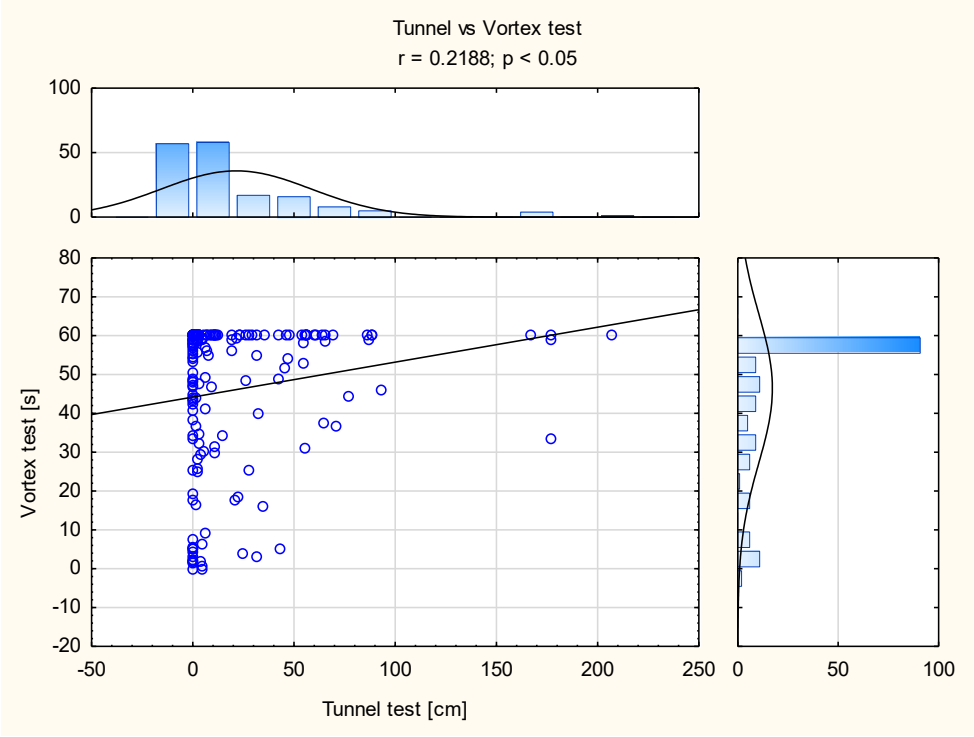

Fig. S1B

4.4 Successful despite poor flight performance: range expansion is associated with enhanced exploratory behavior and fast development

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Successful despite poor flight performance: range expansion is associated with enhanced exploratory behaviour and fast development

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Running head: Factors underlying range expansion

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Abstract

Anthropogenic interference forces species to respond to changing environmental conditions. One possible response is dispersal and concomitant range shifts, allowing individuals to escape unfavourable conditions or to track the shifting climate niche. Range expansions depend on both dispersal capacity and the ability to establish populations beyond the former range. We here compare well-established core populations with recently established edge populations in the currently northward expanding butterfly *Lycaena tityrus*. Edge populations were characterized by shorter development times and smaller size, a higher sensitivity to high temperature, and an enhanced exploratory behaviour. The differences between core and edge populations found suggest adaptation to local climates and an enhanced dispersal ability in edge populations. In particular, enhanced exploratory behaviour may be advantageous in all steps of the dispersal process and may have facilitated the current range expansion. This study describes differences associated with a current range expansion, knowledge which might be useful for a better understanding of species responses to environmental change. We further report on variation between males and females in morphology and flight behaviour, with males showing a longer flight endurance and more pronounced exploratory behaviour than females.

Keywords: Dispersal; global change; flight ability; life-history; local adaptation; *Lycaena tityrus*; range shift.

Introduction

Human-induced environmental change like the current increase in the Earth's mean temperature are expected to strongly affect ecosystems and biodiversity (Hansen *et al.*, 2012; Stanton *et al.*, 2015). Especially changes in temperature may proof to be of utmost importance, because temperature is one of the most important ecological factors affecting, for instance, species performance, survival, and distributions (Overgaard & Sørensen, 2008; Sunday *et al.*, 2011). Facing changing environmental conditions, the long-term survival of all organisms depends on their ability to respond to such changes either by (1) phenotypic plasticity, which allows species to tolerate novel conditions, (2) genetic adaptation, or (3) movement to more suitable habitats often resulting in range shifts (Davis *et al.*, 2005; Parmesan, 2006; Berg *et al.*, 2010; Hofmann & Todgham, 2010). The principal mechanism underlying range shifts is dispersal, often defined as any movements potentially leading to gene flow (Ronce, 2007). It constitutes a key process in the colonization of new areas to track shifting climate niches, is central to the spatial dynamics of populations and metapopulations, and affects patterns of local adaptation (Hanski, 1999; Doebeli & Dieckmann, 2003; Hickling *et al.*, 2006; Ronce, 2007; Baguette *et al.*, 2013).

In general, dispersing individuals do not seem to comprise a random subset of a given population. Rather, dispersers often possess several correlated life-history traits forming a so called dispersal syndrome discriminating between dispersing and non-dispersing conspecifics (Bonte & Saastamoinen, 2012; Stevens *et al.*, 2013). Traits potentially affecting dispersal include morphology, physiology, and overall condition (Bowler & Benton, 2005; Bonte *et al.*, 2012; Therry *et al.*, 2014) as well as behavioural traits such as exploration, aggression, and sociability (Dingemanse *et al.*, 2003;

Duckworth & Badyaev, 2007; Cote *et al.*, 2010b; Ducatez *et al.*, 2012). Given that new populations are likely founded by the most dispersive individuals (cf. Hill *et al.*, 1998; Thomas *et al.*, 1998; Hanski *et al.*, 2002, 2004) and that dispersal-related traits are heritable (Roff & Fairbairn, 2001), more dispersive genotypes are expected to accumulate in recently established edge populations (spatial sorting; Shine *et al.*, 2011). Life-history traits associated with dispersal ability are often affected by environmental conditions. For instance, suboptimal conditions (e.g. during development) may decrease the investment into costly dispersal traits (Bonte *et al.*, 2008). On the other hand, such conditions may increase dispersal ability or at least the willingness to do so as an adaptive response for dealing with detrimental conditions (e.g. Monaghan, 2008). Environmental effects on dispersal ability / tactics have been repeatedly demonstrated through alterations in body composition or behaviour (Bonte *et al.*, 2008; Saastamoinen *et al.*, 2010; Van den Heuvel *et al.*, 2013).

However, dispersal ability is only one facet of range expansions. The other crucial component is the ability to establish populations beyond the current range once suitable habitats have been reached by dispersing individuals. For instance, temperature variation along geographical gradients may require an ‘optimization’ of phenotypic values and thus local adaptation (Hoffmann *et al.*, 2002; Castañeda *et al.*, 2005). Hence, successful range expansions may further depend on a wide tolerance against environmental stressors and / or the evolutionary potential of a given species. Consequently, phenotypic differences between recently established edge and long-established core populations are expected in traits related to dispersal, but also in other traits such as thermal tolerance (Hanski *et al.*, 2004; Calosi *et al.*, 2008; Lindstrom *et al.*, 2013). This is due to differences in selection pressures but may also result from

differences in the genetic structure of core and edge populations (Volis *et al.*, 1998; Purves, 2009; Mägi *et al.*, 2011). Such differentiation may also include phenotypic plasticity in those traits (Otaki *et al.*, 2010; Pichancourt & van Klinken, 2012).

Depending on dispersal and establishing capacity some species may benefit while others may suffer from current climate change (Pimm, 2001; Thomas *et al.*, 2001; Klockmann *et al.*, 2016). Many temperate zone ectotherms may actually benefit from rising temperatures, because conditions at northern margins may become more suitable (Pimm, 2001; Deutsch *et al.*, 2008; Bestion *et al.*, 2015). One such species is the currently northward expanding butterfly *Lycaena tityrus* (Brunzel *et al.*, 2008; Settele *et al.*, 2008; Klockmann *et al.*, 2016), which has colonized north-eastern Estonia very recently. We here compared replicated core (Germany) and recently established edge (Estonia) populations of this temperate-zone butterfly. In order to explore variation in dispersal ability and life history indicative of local adaptation, we investigated a variety of traits ranging from development, morphology, physiology, and flight endurance through to behaviour. As differences in dispersal ability can be related to flight performance (Ducatez *et al.*, 2012; Legrand *et al.*, 2015) and / or morphology (Berwaerts & van Dyck, 2004), we focused on traits potentially associated with flight and dispersal ability, including wing size, body mass, thorax-abdomen ratio, wing aspect ratio, wing loading, and storage reserves (Betts & Wootton, 1988; Berwaerts *et al.*, 2002). Many of these traits might also show environmental-induced plasticity, which may in turn feedback on dispersal ability. We here tested the following hypotheses: (1) Estonian edge populations will differ from German core populations in traits indicative of local adaptation to cooler conditions and shorter vegetation period. Specifically, we predict a more rapid development in edge populations. (2) Edge populations show

increased dispersal ability, evidenced by a higher flight endurance and enhanced exploratory behaviour. Increased dispersal ability is associated with morphological and / or physiological differentiation among edge and core populations.

Material and methods

Study organism

Lycaena tityrus (Poda, 1761) is a widespread temperate-zone butterfly with a range from Western Europe to central Asia (Ebert & Rennwald, 1991). It is currently expanding its range towards higher latitudes and altitudes, which is assumed to be largely driven by anthropogenic climate change (Brunzel *et al.*, 2008; Settele *et al.*, 2008). The species is bivoltine with two discrete generations per year in most parts of its range, although populations with one or three generations per year occur. Overwintering takes place as half-grown larvae. The principal larval host-plant is *Rumex acetosa L*. (Polygonaceae), but some congeneric *Rumex* species are utilized as well (Ebert & Rennwald, 1991; Tolman & Lewington, 1998; Settele *et al.*, 2008). Adults are nectar feeders, using a wide array of species including several Asteraceae (Ebert & Rennwald, 1991).

The butterflies used in this study were collected in three replicated populations each in north-eastern Germany and Estonia. While the German populations were located within the core of the distribution area, the Estonian ones reflect currently expanding edge populations, which were probably established within the last 5 years (T. Esperk & T. Tammaru, pers. comm.). The first records of *L. tityrus* in Estonia stem from the end of the 20th century (Õunap & Tartes, 2014). In total 69 and 56 freshly eclosed,

fecund females were caught in May and June 2016 in Germany and Estonia, respectively (Germany: Guest 54°02'56.4" N, 13°26'18.3" E, n = 18; Ueckermünde 53°44'52.6" N, 14°15'28.3" E, n = 25; Waren 53°30'13.3" N, 12°42'31.5" E, n = 26; Estonia: Karepa 59°32'39.3" N, 26°25'34.4" E, n = 22; Kasispea 59°35'05.6" N, 25°46'21.9" E, n = 12; Mahu 59°31'09.8" N, 26°42'58.5" E, n = 22). All these populations represent bivoltine lowland populations. Both regions differ substantially in annual mean temperature (Germany: 8.2°C, Estonia: 4.8°C), but less so in annual precipitation (Germany: 560 mm, Estonia: 606 mm). The temperature differences prevail throughout the time during which the first generation develops and reproduces (April: 6.6 vs 3.6°C, May: 11.5 vs 10.3°C June: 15.4 vs 14.9°C; climate-data.org). All females were transferred to a climate chamber at Greifswald University for egg laying.

Experimental design

Caught females were kept at 26°C, 60% relative humidity, and a L18:D6 photoperiod. For oviposition, females were placed separately in translucent 1 L plastic pots covered with gauze, and were provided with *R. acetosa* (oviposition substrate), fresh flowers (*Achillea millefolium L., Leucanthemum vulgare*, *Hypochaeris radicata* L., *Tanacetum vulgare L.*), water, and a highly concentrated sucrose solution (20 vol%) for adult feeding. Deposited eggs were collected daily and transferred, separated by female, to small plastic boxes. For each female, resulting larvae (maximum number of 20 per female) were divided into two treatment groups. The first group of larvae was reared under the same conditions used for oviposition, while the second group experienced cooler conditions (18°C, 60% relative humidity, L18:D6 photoperiod). All individuals were kept at their respective temperature treatment throughout. Larvae were individually reared in small plastic boxes (125 ml) and had access to food in ample

supply until pupation. Boxes contained moistened filter paper and fresh cuttings of *R. acetosa* and *R. acetosella*. Pupae were weighed on the day following pupation. For each individual we recorded larval and pupal development time and additionally calculated larval growth rate (pupal mass divided by larval time). Resulting butterflies were kept individually and were provided with moistened cotton for drinking. One day-old butterflies were first subjected to a behavioural experiment testing the exploratory behaviour and on the following day to a flight endurance test (see below). Exploratory behaviour, relying on spontaneous flight, was tested in a climatic chamber at 26°C and 60% relative humidity. Flight endurance (forced flight), in contrast, was tested at 22°C and 60% relative humidity (note that no differences were found between 22 and 26°C in pilot experiments). All butterflies could acclimatise for 1 hour to the conditions in the experimental chambers prior to the experiments. Unfortunately, a high number of Estonian larvae raised at 18°C entered diapause which caused low sample sizes in the respective treatment groups.

Exploratory behaviour

This test was designed to investigate the individual's exploratory behaviour towards a food source in an unknown experimental setup. Each individual was placed singly into the back-right corner of a cage (50 x 50 x 50 cm). A food source consisting of flowering plants (see above) and two leaves of the host plant (*R. acetosa*) was placed in a small vial in the front-left corner of the cage. Butterflies were released and observed for longest 90 minutes. It was scored if the butterfly reached the food source or not. Afterwards, butterflies were kept individually at their rearing conditions until the start of the flight endurance test the next day.

Flight endurance test

Butterflies were tested for flight endurance using a well-established assay (cf. Ducatez *et al.*, 2012, 2013). This test was designed to detect individual differences in flight endurance under stressful conditions and has been shown to be correlated with other mobility measures and dispersal (Ducatez *et al.* 2012, Legrand *et al.* 2015). Each individual was placed into a plastic chamber (30 x 16 x 14 cm), which was perforated at its base and fixed to a rapid agitator (IKA Vortex 4 digital). After a habituation period of 30 seconds, the vortex was switched on to strongly shake the chamber, preventing the butterfly from holding on to the walls. This method forces the butterflies to fly during the test, as they were otherwise lying uncomfortably on the bottom of the shaking chamber. The time an individual butterfly spent flying was recorded for 60 seconds, with higher values reflecting a higher flight endurance. After the endurance test, butterflies were frozen for later analyses of potentially flight-related parameters.

Morphological and physiological analyses

To test for associations between exploratory behaviour or flight endurance and other traits, we measured a couple of morphological and physiological traits. First, adult body mass was determined to the nearest 0.01 mg (Sartorius CPA225D). Then, wings, heads and legs were removed. Thorax and abdomen were separated and afterwards weighed. Abdomen fat content was measured following Fischer *et al.* (2003), but using the less poisonous acetone instead of dichloromethane. Abdomens were first dried to constant weight for two days at 70°C. Abdomen dry mass was measured. Afterwards, fat was extracted twice for 48 h each, using 1 ml of acetone (C_3H_6O) for each abdomen. Solutions were exchanged between both fat extractions. Then, abdomens were again dried for two days at 70°C, after which the fat-free dry mass was measured. Absolute

(mg) and relative (%) abdomen fat content were determined as the mass difference between abdomen dry mass and the remaining dry mass after the two fat extractions. Forewing area and length (from basis to apex) were measured using digital images of left forewings (captured from ventrally with a digital camera mounted on a stereo microscope) and NIS elements software. Wing loading was calculated as total body mass divided by forewing area, and wing aspect ratio as 4 x forewing length^2 divided by the forewing area (Berwaerts *et al.*, 2002). Thorax-abdomen ratio was calculated by dividing thorax through abdomen mass.

Statistical analyses

Data on developmental, morphological, and physiological traits were analysed with general linear mixed models (GLMs), using country (origin), developmental temperature and sex as fixed factors, and population (nested within country) as a random effect. Due to small sample sizes in the 18°C treatment groups for Estonian individuals, the factor family was only included in the additional models (see below). Data on abdomen mass, thorax-abdomen ratio, and absolute and relative fat content were LN transformed prior to analysis to meet GLM requirements. Data on exploratory behaviour (reaching of food source: yes / no) were analysed using generalized linear models (GzLMs) with a binomial error distribution and logit-link function, using the same factors as above.

To additionally test for the impact of continuous variables (morphology, physiology) on flight behaviour and endurance, we first performed principal component analyses (PCAs) owing to strong inter-correlations among traits. PCAs were calculated separately for males and females, as sexes differed strongly in a variety of traits. We

used the first four principal components (PCs) for further analyses, having Eigenvalues between 4.3 and 1.1 for males and between 5.0 and 1.0 for females (cf. Table 1). Thus, all PCs explaining ≥ 10% of the variance were included. We then constructed another set of models by including the respective PCs and the results of the behavioural or flight endurance test as covariates to the models mentioned above, followed by a stepwise backwards elimination of non-significant factors.

Due to small sample sizes in the 18°C treatment groups for Estonian individuals, we additionally calculated models (same structure as above, but additionally including family nested within population and country as a random factor) exclusively based on (1) the animals reared at 26°C, thus focusing on differences across countries (appendix A1), and (2) on the animals from Germany, thus focusing on differences across developmental temperature (18°C versus 26°C, appendix A2). As these additional analyses confirmed the results obtained from the above full-factorial models, only the latter ones are presented here (for additional models please see appendix A1 and A2). All statistical tests were performed with Statistica 8.0 (Tulsa, StatSoft, OK). All means are given ± 1 SE.

Results

Developmental and morphological traits

Country significantly affected larval and pupal development time, pupal mass, adult body mass, wing length, wing area and aspect ratio (Table 2). German compared with Estonian individuals had longer larval and pupal development times, higher pupal and adult body masses, larger wings, and lower wing aspect ratios (Fig. 1). Differences

among populations were significant for all traits except for wing length, wing area and aspect ratio (cf. appendix A3). Developmental temperature significantly affected all traits except for thorax mass, relative fat content, wing length and area, indicating that higher temperatures during development led to a shorter development time, higher growth rate, higher pupal but lower abdomen and adult body mass, higher thorax-abdomen ratio, lower absolute fat content, wing loading and aspect ratio (Table 2, Fig. 1). Significant sex differences were found for all traits except from pupal mass and wing length, indicating that females had on average a longer development time, lower growth rate, higher absolute fat content, wing loading, abdomen and concomitantly total body mass, lower thorax mass, thorax-abdomen ratio, relative abdomen fat content, and wing aspect ratio, and a larger wing area than males (Table 2, Fig. 1).

Country by temperature interactions were significant for all traits except for larval time, thorax-abdomen ratio, and wing length. They show that temperature effects on pupal mass were restricted to German populations but absent in Estonian ones (Fig. 1c). Similar patterns of positive effects of the higher temperature on German but negative ones on Estonian individuals were found for thorax mass (Fig. 1g), wing area (Fig. 1l), and relative fat content (Fig. 1j). For adult body mass, the significant interaction indicates that the difference between temperatures was more pronounced in Estonian compared with German individuals (Fig. 1e). The same pattern was found for abdomen mass (Fig. 1f), absolute fat content (Fig. 1i), and wing loading (Fig. 1m). For pupal time, the difference between temperatures was more pronounced in German than in Estonian individuals (Fig. 1b), which was also the case in aspect ratio (Fig. 1n).

Country by sex interactions were significant for pupal mass, thorax mass, and wing length and area. Accordingly, sexual differences in pupal mass were restricted to German populations (Fig. 1c). A similar pattern was found for wing length (Fig. 1k). For thorax mass, the interaction shows that the difference between sexes was more pronounced in German than in Estonian individuals (Fig. 1g). For wing area, in contrast, the difference between the sexes was more pronounced in Estonian compared with German individuals (Fig. 1l).

Temperature by sex interactions were significant for pupal mass, pupal time, and wing length. For pupal mass and pupal time, the difference between the sexes was more pronounced at 26°C than at 18°C (Fig. 1b, 1c). The opposite pattern though was found for wing length (Fig. 1k). The three-way interaction, finally, was significant for wing length only. It shows that differences among countries persisted in all treatment groups except for females at 18°C (Fig. 1k).

Exploratory behaviour and flight endurance

Exploratory behaviour was significantly affected by the factors country, sex, and temperature (Table 3a). Estonian butterflies, males, and individuals that were raised at 26°C were more successful in reaching the food source (Fig. 2a). Including principal components and the flight endurance in the vortex as covariates revealed significant effects of the country by temperature interaction, PC1 and the vortex test results for males, and of temperature for females (Table 3a). The country by temperature interaction in males shows that the temperature effect on exploratory behaviour was more pronounced in Estonian (18°C: 41.7 vs 26°C: 70.1%) than in German males (18°C: 38.0 vs 26°C: 64.5%). In males, flight behaviour was negatively correlated with

PC1 (beta -0.11 ± 0.05) and the vortex test results (beta -0.14 ± 0.04). PC1 was most strongly (positively) related to body mass (Table 1a), meaning that a lower body mass increased exploratory behaviour in males. Furthermore, males that were more exploratory performed worse in the vortex. The temperature effect found in females reflects the above pattern that individuals having been raised at 26°C were more exploratory.

Flight endurance was significantly affected by the factors population, sex, and the interactions between country and sex and temperature and sex (Table 3b). Males showed a higher flight endurance than females (Fig. 2b). The interaction between country and sex though shows that the higher flight endurance of males was largely restricted to German animals. The temperature by sex interaction indicates that males had a higher flight endurance when raised at 18°C only, while for individuals raised at 26°C values were very similar (Fig. 2b).

Including principal components and exploratory behaviour as covariates revealed significant effects of population, temperature, PC1, PC3, and exploratory behaviour for males, and of temperature, PC1, and PC4 for females (Table 3b). Males had a higher flight endurance when having been raised at 18°C rather than 26°C. Flight endurance was positively correlated with PC1 (beta 0.29 ± 0.04) and PC3 (beta 0.11 ± 0.04). PC1 was most strongly (positively) correlated with body mass (Table 1a), meaning that a higher body mass increased flight endurance. PC3 was most strongly (positively) correlated with thorax-abdomen ratio (Table 1a), showing that a high thorax-abdomen ratio led to a higher flight endurance in males. Males that showed reduced exploratory behaviour performed better in the vortex. Females, in contrast to males, had a higher

flight endurance when having been raised at 26°C rather than 18°C. Flight endurance was negatively correlated with PC1 (beta -0.17 ± 0.04) and PC4 (beta -0.11 ± 0.04). PC1 in turn was most strongly (negatively) correlated with adult body mass (Table 1b), meaning that a high body mass led to a higher flight endurance. PC4 was most strongly (negatively) correlated with aspect ratio (Table 1b), showing that a higher aspect ratio increased flight endurance in the vortex. Country also tended to affect flight endurance of females, showing that Estonian females tended to perform better than German females.

Discussion

We start our discussion with the effects of developmental temperature, country (origin), and sex on variation in developmental and morphological traits and continue with exploratory behaviour and flight endurance, as the former may causally underlie variation in the latter.

Developmental and morphological traits

Interestingly, Estonian individuals had shorter development times accompanied by lower body size compared with German individuals. Short development time typically evolves when a minimum size needs to be reached within a limited time period (Case, 1978; Arendt, 1997). More severe time constraints in Estonia as compared with Germany certainly arise from a shorter growing season with increasing latitude (Blanckenhorn, 1997). Thus, the cooler conditions experienced in Estonia likely selected for shorter development time in order to compensate for the limitations imposed by the cooler environmental conditions. Such local adaptation is especially

expected in insects exhibiting more than one generation per year, driven by the need to fit in yet another generation (Fischer & Karl, 2010). Although local adaptation has been repeatedly shown in butterflies (e.g. Ayres & Scriber, 1994; Phillimore *et al.*, 2012), the case reported here seems interesting as differences may have evolved within a relatively short period of time, as the sampled Estonian populations were established only recently. We also found negative effects of the higher developmental temperature on thorax mass, wing area, and relative fat content in Estonian butterflies, while German individuals benefitted from warmer conditions. This might further support the notion of local adaptation to the cooler Estonian climate or at least indicate a loss of adaptation to warmer conditions. Note furthermore the higher incidence of diapause induction in Estonian animals at the cooler temperature (Estonia: 80%; Germany: 5%), suggesting a higher threshold for direct development in Estonian individuals. Taken together, the above lines of evidence suggest that Estonian individuals are relatively better adapted to cooler and German ones to warmer environmental conditions.

Regarding temperature effects, the higher temperature reduced development times and adult body mass (following the temperature-size rule), as expected for an ectothermic organism (Nylin, 1989; Atkinson, 1994; Blanckenhorn, 1997; Fischer & Fiedler, 2000). Shorter development times are generally achieved through enhanced growth and metabolic rates at higher temperatures (Karl & Fischer, 2008). Higher abdomen and thus adult body mass (resulting in a higher absolute fat content and wing loading, but a lower thorax-abdomen ratio) at lower temperatures typically result from behavioural and physiological mechanisms like an increased food intake and a more efficient conversion of the ingested food into body matter (Karl & Fischer, 2008; Fischer & Karl, 2010). Note though that, contrary to the temperature-size rule (Nylin, 1989;

Atkinson, 1994), pupal mass was higher when being reared at higher temperatures (except for the pupal mass of Estonian females), which may have been caused by the higher growth rates. As these entail increased physiological costs that lead to high weight losses during metamorphosis (Gotthard *et al.*, 1994; Fischer *et al.*, 2005; Fischer & Karl, 2010), the expected pattern nevertheless arose in the adult stage.

The sex differences in developmental, morphological, and physiological traits found in this study are in line with previous studies on (*Lycaena*) butterflies (e.g. Karl *et al.*, 2008; Fischer & Karl, 2010; Saastamoinen *et al.*, 2012). Male butterflies had shorter development times and higher growth rates (and an accordingly earlier adult emergence) to enhance mating opportunities (selection for protandry; Wiklund & Fagerström, 1977; Fischer & Fiedler, 2000; Karl & Fischer 2008), but were lighter as adults compared with females (fecundity selection in females; Honek, 1993; Blanckenhorn, 2000). In German males, this pattern also applied to pupal mass, suggesting that shorter development times decreased pupal weight. Interestingly, pupal mass of Estonian individuals was almost identical in males and females, perhaps reflecting the strong time pressure to develop fast in both sexes. Regarding flight-related traits, males had a lower wing loading but a higher thorax-abdomen ratio, wing aspect ratio, and relative fat content, traits that are often associated with increased flight ability, acceleration capacity, and maneuverability (Betts & Wootton, 1988; Berwaerts *et al.*, 2002, 2006; Van Dyck & Wiklund, 2002; Saastamoinen *et al.*, 2012).

Exploratory behaviour and flight endurance

Exploratory behaviour, tested here as the ability to locate a food source in an unknown experimental setup, was more pronounced in butterflies from expanding edge

populations (Estonia), though effects were largely restricted to individuals from the warmer temperature. However, good exploration skills may help during all steps of the dispersal process, beginning with the actual decision to emigrate via effective movement in the transition phase through to the location of suitable habitats (Fraser *et al.*, 2001; Ronce, 2007; Cote *et al.*, 2010a; Ducatez *et al.*, 2012). Within populations, more explorative individuals can have a higher dispersal propensity (Cote *et al.*, 2010a; Ducatez *et al.*, 2012), which may lead to an accumulation of bolder individuals in edge populations as indicated here. Interestingly, butterflies reared at 26°C were more exploratory compared with butterflies reared at 18°C. Although direct behavioural responses to the thermal conditions experienced during development are poorly documented, temperature might be a reliable indicator of optimal dispersal tactics later in life (Tautz *et al.*, 2003; Bonte *et al.*, 2008). Thus, having experienced beneficial conditions during development may indicate suitable conditions for dispersal and thus increase exploratory behaviour. This might be especially relevant in Estonia due to stronger temperature constraints, which may explain the above interaction.

An alternative explanation for the positive effect of higher temperatures could be that the butterflies were more attracted to the food source. Higher temperatures (and accordingly higher metabolic rates) during development and in the adult stage prior to the experiment could have increased their need for drinking and feeding. This would be in line with the morphological differences found, showing that butterflies reared at the higher temperature had a lower body mass and fat content. This notion is additionally supported by the negative correlation between exploratory behaviour and PC1 in males, demonstrating that lower body masses increased exploratory behaviour. Furthermore, males that were more explorative had a shorter flight endurance and vice

452 versa. The overall weak effects of morphological and physiological traits on exploratory
453 behaviour suggest that it is not closely related to the individuals' condition, but rather
454 a behavioural property which is modulated by environmental circumstances (e.g.
455 developmental temperatures).
456
457 Males were in general more exploratory than females, which might be a result of
458 differential selective pressures (Gilchrist, 1990; Van Dyck & Wiklund, 2002; Merckx &
459 Van Dyck, 2005; Berwaerts *et al.*, 2006). After emergence, males try to quickly
460 establish a territory to enhance mating opportunities (Davies, 1978; Fischer & Fiedler,
461 2000; Saastamoinen *et al.*, 2012), which could explain their more exploratory
462 behaviour evidenced by a faster location of a food source.
463
464 German males showed a higher flight endurance in the vortex test than Estonian
465 males. Mechanistically, this difference might be linked to a higher body mass and size
466 of German individuals. Interestingly, Estonian females tended to show the opposite
467 pattern, viz. a higher flight endurance compared with German females. Likewise,
468 Hanski *et al.* (2004) found in the butterfly *Melitaea cinxia* that females from newly
469 established and isolated populations were most dispersive. Males showed a higher
470 flight endurance than females, but only when having been raised at 18°C. As males
471 have a lower wing loading but a higher thorax-abdomen ratio, a higher flight endurance
472 of males was expected (Wickman, 2009; Saastamoinen *et al.*, 2012). In contrast to
473 exploratory behaviour, morphological traits seemed to have a stronger impact on flight
474 endurance (significant impacts of 2 PCs for each males and females), which suggests
475 that it is more tightly linked to morphology than is the actual behaviour (Van Dyck &
476 Wiklund, 2002; Berwaerts *et al.*, 2006). In both sexes, flight endurance was positively

related to body size and therefore storage reserves, which are used to fuel flight and reflect a good condition in general (Zera *et al.*, 1998; Berwaerts *et al.*, 2002; Karl & Fischer, 2008). In males, we also found a positive influence of a higher thorax-abdomen ratio, which is generally assumed to enhance flight performance and acceleration capacity (Marden, 1989; Thomas *et al.*, 1998; Berwaerts *et al.*, 2002). In females, flight endurance was additionally related to high wing aspect ratios, reflecting increased flight ability (Berwaerts *et al.*, 2002; Berwaerts & Van Dyck, 2004). Population effects were significant in most cases, indicating genetic variation among and within populations in the traits investigated (Finger *et al.*, 2009; Klockmann *et al.*, 2016). This did not interfere with any of the conclusions presented here.

Conclusions

We here show that sexes differed substantially in developmental, morphological, and physiological traits, being arguably the result of differential selective pressures (e.g. Gilchrist, 1990; Berwaerts *et al.,* 2006). In general, males showed traits associated with increased flight ability, which resulted in a higher flight endurance in the vortex and enhanced exploratory behaviour, which might be helpful to quickly establish a territory (Fischer & Fiedler, 2000; Saastamoinen *et al.,* 2012). In the current era of climate change, many temperate-zone ectotherms might actually benefit from rising global temperatures (Deutsch *et al.*, 2008). One such example is the currently northward expanding butterfly *L. tityrus*, which has recently expanded in north-eastern direction and thereby colonized Estonia. The colonization process in turn obviously depends on dispersal ability, and may warrant local adaptation to new environmental conditions (Doebeli & Dieckmann, 2003). Despite having colonized Estonia only very recently, we detected several differences between edge and core populations

indicative of local adaptation. Estonian edge as compared with German core populations were characterized by shorter development times and smaller size, a higher sensitivity to high temperature, a higher diapause incidence, and enhanced exploratory behaviour. These differences seem to partly reflect adaptations to the cooler Estonian climate and the concomitantly short vegetation period (or a loss of adaptation to warmer conditions), while others, namely enhanced exploratory behaviour, may indicate increased dispersal capacity which may have partly facilitated the current range expansion. While the time frame available may not have been sufficient for *in situ* adaptation, it is currently unclear whether the differences between core and edge populations reflect clinal variation (assuming that the populations from which Estonia was colonized already differed from those in Germany) or an accumulation of cold-adapted and / or more dispersive genotypes in edge populations. Note that butterfly females mate quickly after adult eclosion (Hill *et al.*, 1999) and lay at least a proportion of their eggs after dispersal, hence enabling colonization (Stevens *et al.*, 2012). Also, we cannot rule out a contribution of parental effects, which may comprise part of adaptive responses to environmental variation (Wolf *et al.*, 1998; Mattila & Hanski, 2014). In summary, this study provides insights into the factors associated with range expansion, knowledge which might be useful for a better understanding of species responses to environmental change.

Conflict of interests

The authors have no conflict of interests to declare.

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758

Tables

Table 1 Results of principal component analyses for males (a) and females (b) including Eigen values, percentage of the variance explained, cumulative percentage of the variance explained, and *r*-values of correlations with continuous variables (rows 4-13) for principal components 1-4. *r*-values > 0.7 in bold.

Table 1a

No	Parameter	PC1	PC2	PC3	PC4
1	Eigen value	4.3	2.0	1.8	1.1
2	Variance explained (%)	43.3	19.7	17.6	11.2
3	Cumulative variance (%)	43.3	63.0	80.5	91.8
4	Adult body mass	**0.989**	0.038	-0.042	0.065
5	Thorax mass	**0.795**	-0.070	-0.582	0.044
6	Abdomen mass	**0.901**	0.099	0.272	0.081
7	Thorax-abdomen ratio	-0.093	0.167	**0.903**	0.017
8	Fat (mg)	0.231	**-0.950**	0.183	-0.014
9	Fat (%)	0.068	**-0.962**	0.248	-0.037
10	Wing Length	**0.793**	0.206	0.357	-0.282
11	Wing area	**0.803**	0.194	0.377	0.168
12	Wing loading	**0.750**	-0.123	-0.400	-0.057
13	Wing aspect ratio	0.034	0.041	-0.018	**-0.998**

768 Table 1b

No	Parameter	PC1	PC2	PC3	PC4
1	Eigen value	5.1	2.0	1.3	1.0
2	Variance explained (%)	51.2	19.8	12.5	10.5
3	Cumulative variance (%)	51.2	71.0	83.5	94.0
4	Adult body mass	**-0.983**	0.118	-0.015	0.075
5	Thorax mass	**-0.943**	-0.069	-0.275	0.146
6	Abdomen mass	**-0.809**	0.363	0.304	-0.043
7	Thorax-abdomen ratio	0.559	0.398	0.605	-0.239
8	Fat (mg)	-0.505	**-0.790**	0.291	-0.179
9	Fat (%)	-0.242	**-0.870**	0.353	-0.235
10	Wing Length	**-0.798**	0.381	0.145	-0.349
11	Wing area	**-0.816**	0.345	0.352	-0.006
12	Wing loading	**-0.827**	-0.116	-0.343	0.117
13	Wing aspect ratio	0.028	0.098	-0.496	**-0.860**

769

770 **Table 2** General linear mixed model results for the effects of country, temperature, sex
771 (fixed factors), and population (nested within country; random factor) on developmental
772 and morphological traits in *Lycaena tityrus*. Models were constructed by a stepwise
773 backwards elimination of non-significant factors. Eta-squared represents effect size.
774 Significant *P*-values are given in bold.

775

Larval time	***MS***	***DF***	***Eta²***	***F***	***P***
Country	769.3	1, 5	0.92	49.6	**0.0014**
Population (Country)	20.8	4, 1483	0.02	7.4	**< 0.0001**
Temperature	99102.6	1, 1483	0.96	35374.3	**< 0.0001**
Sex	1635.3	1, 1483	0.28	583.7	**< 0.0001**
Error	2.8	1483			
Pupal time	***MS***	***DF***	***Eta²***	***F***	***P***
Country	128.1	1, 5	0.94	97.4	**< 0.0001**
Population (Country)	3.0	4, 1481	0.02	8.8	**< 0.0001**
Temperature	7371.4	1, 1481	0.93	21293.6	**< 0.0001**
Sex	6.8	1, 1481	0.01	19.6	**< 0.0001**
Country*Temperature	34.9	1, 1481	0.06	100.7	**< 0.0001**
Temperature*Sex	9.7	1, 1481	0.02	28.2	**< 0.0001**
Error	0.4	1481			
Pupal mass	***MS***	***DF***	***Eta²***	***F***	***P***
Country	9115	1, 5	0.717	13.7	**0.0121**
Population (Country)	1605	4, 1479	0.029	11.1	**< 0.0001**
Temperature	1131	1, 1479	0.005	7.8	**0.0053**
Country*Temperature	2670	1, 1479	0.012	18.4	**< 0.0001**
Country*Sex	2014	1, 1479	0.009	13.9	**0.0002**
Temperature*Sex	795	1, 1479	0.002	5.5	**0.0194**
3-way-interaction	476	1, 1479	0.004	3.3	0.0704
Error	145	1479			

776

777 Table 2 continued

Growth rate	***MS***	***DF***	***Eta²***	***F***	***P***
Country	1.7	1, 5	0.05	0.2	0.6555
Population (Country)	10.3	4, 1482	0.84	12.3	**< 0.0001**
Temperature	6363.3	1, 1482	0.03	7584.7	**< 0.0001**
Sex	187.9	1, 1482	0.13	224.0	**< 0.0001**
Country*Sex	0.1	1, 1482	< 0.01	0.2	0.6880
Error	0.8	1482			
Adult body mass	***MS***	***DF***	***Eta²***	***F***	***P***
Country	607.9	1, 5	0.637	13.4	**0.0070**
Population (Country)	87.8	4, 1360	0.013	4.4	**0.0016**
Temperature	571.5	1, 1360	0.167	28.5	**< 0.0001**
Sex	5459.9	1, 1360	0.007	272.7	**< 0.0001**
Country*Temperature	189.5	1, 1360	0.021	9.5	**0.0021**
Error	20.0	1360			
Abdomen mass	***MS***	***DF***	***Eta²***	***F***	***P***
Country	0.50	1, 5	0.46	5.6	0.0520
Population (Country)	0.19	4, 1360	0.02	5.8	**0.0001**
Temperature	3.41	1, 1360	0.07	106.5	**< 0.0001**
Sex	109.29	1, 1360	0.72	3415.5	**< 0.0001**
Country*Temperature	0.31	1, 1360	0.01	9.5	**0.0021**
Error	0.03	1360			
Thorax mass	***MS***	***DF***	***Eta²***	***F***	***P***
Country	17.4	1, 5	0.117	0.7	0.4406
Population (Country)	58.3	4, 1359	0.033	11.4	**< 0.0001**
Sex	749.0	1, 1359	0.098	147.1	**< 0.0001**
Country*Temperature	54.8	1, 1359	0.016	10.8	**< 0.0001**
Country*Sex	118.9	1, 1359	0.017	23.3	**< 0.0001**
Error	5.1	1359			

778

779 Table 2 continued

Thorax-abdomen ratio	***MS***	***DF***	***Eta²***	***F***	***P***
Country	0.86	1, 5	0.36	2.6	0.1741
Population (Country)	0.45	4, 1360	0.02	6.9	**< 0.0001**
Temperature	20.30	1, 1360	0.19	311.0	**< 0.0001**
Sex	401.29	1, 1360	0.82	6149.0	**< 0.0001**
Country*Sex	0.16	1, 1360	< 0.01	2.4	0.1179
Error	0.07	1360			
Fat (mg)	***MS***	***DF***	***Eta²***	***F***	***P***
Country	1.9	1, 5	0.18	0.3	0.5854
Population (Country)	15.2	4, 1313	0.11	27.1	**< 0.0001**
Temperature	9.6	1, 1313	0.01	17.0	**< 0.0001**
Sex	62.4	1, 1313	0.07	110.9	**< 0.0001**
Country*Temperature	10.0	1, 1313	< 0.01	17.7	**< 0.0001**
Error	0.6	1313			
Fat (%)	***MS***	***DF***	***Eta²***	***F***	***P***
Country	239.6	1, 5	0.080	0.4	0.5610
Population (Country)	1711.7	4, 1314	0.090	32.4	**< 0.0001**
Temperature	154.5	1, 1314	0.002	2.9	0.0875
Sex	7459.5	1, 1314	0.097	141.2	**< 0.0001**
Country*Temperature	229.3	1, 1314	0.003	4.3	**0.0374**
Error	52.8	1314			
Wing length	***MS***	***DF***	***Eta²***	***F***	***P***
Country	65.2	1, 1364	0.118	181.7	**< 0.0001**
Country*Sex	12.6	1, 1364	0.025	35.2	**< 0.0001**
Temperature*Sex	2.3	1, 1364	0.005	6.3	**0.0121**
3-way Interaction	1.8	1, 1364	0.004	4.9	**0.0267**
Error	0.4	1364			

780

781 Table 2 continued

Wing area	***MS***	***DF***	***Eta²***	***F***	***P***
Country	7818	1, 1364	0.11	170.5	**< 0.0001**
Sex	12738	1, 1364	0.17	277.7	**< 0.0001**
Country*Sex	340	1, 1364	0.01	7.4	**0.0065**
Country*Temperature	1511	1, 1364	0.02	33.0	**< 0.0001**
Error	46	1364			
Wing loading	***MS***	***DF***	***Eta²***	***F***	***P***
Country	17.1	1, 5	0.043	0.2	0.6671
Population (Country)	200.5	4, 1360	0.057	20.6	**< 0.0001**
Temperature	957.5	1, 1360	0.067	98.3	**< 0.0001**
Sex	854.7	1, 1360	0.061	87.7	**< 0.0001**
Country*Temperature	63.0	1, 1360	0.005	6.5	**0.0111**
Error	9.7	1360			
Wing aspect ratio	***MS***	***DF***	***Eta²***	***F***	***P***
Country	1.5	1, 5	0.010	13.7	**0.0002**
Temperature	2.5	1, 1364	0.016	22.6	**< 0.0001**
Sex	262.8	1, 1364	0.632	2342.0	**< 0.0001**
Country*Temperature	1.2	1, 1364	0.008	11.0	**0.0010**
Error	0.1	1364			

782

Table 3 Results of a generalized linear model (exploratory behaviour, a) and a general linear mixed model (flight endurance in a vortex, b) for the effects of country, temperature, sex (all fixed), and population (nested within country; random) in the butterfly *Lycaena tityrus* (models 1). Second models were constructed by additionally including PCs 1-4 and the results of the exploratory behaviour or flight endurance test as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. The latter models were constructed separately for males and females, owing to strong variation in morphological traits among the sexes. Odds ratio (a) and Eta-squared (b) represent effect sizes. Significant *P*-values are given in bold.

Table 3a

Model 1	***DF***	***Odds ratio***	***Wald Chi²***	***P***
Country	1	1.3	4.1	**0.0440**
Temperature	1	2.7	55.8	**< 0.0001**
Sex	1	0.4	43.8	**< 0.0001**
Model 2 Males	***DF***	***Odds ratio***	***Wald Chi²***	***P***
Country*Temperature	3		23.9	**< 0.0001**
PC1	1	1.2	5.3	**0.0218**
Flight endurance	1	1.0	10.7	**0.0011**
Model 2 Females	***DF***	***Odds ratio***	***Wald Chi²***	***P***
Temperature	1	2.8	28.6	**< 0.0001**

795 Table 3b

Model 1	***MS***	***DF***	***Eta²***	***F***	***P***
Country	882	1, 5	0.149	0.8	0.4086
Population (Country)	1450	4, 1354	0.016	5.6	**0.0002**
Sex	5708	1, 1354	0.016	22.1	**< 0.0001**
Country*Sex	3816	1, 1354	0.011	14.8	**0.0001**
Temperature*Sex	3953	1, 1354	0.022	15.3	**< 0.0001**
Error	258	1354			
Model 2 Males	***MS***	***DF***	***Eta²***	***F***	***P***
Country	52	1, 5	0.013	0.1	0.7543
Population (Country)	910	4, 551	0.027	3.8	**0.0043**
Temperature	4583	1, 551	0.034	19.4	**< 0.0001**
PC1	6470	1, 551	0.047	27.3	**< 0.0001**
PC3	5623	1, 551	0.041	23.8	**< 0.0001**
Exploratory behaviour	3093	1, 551	0.023	13.1	**0.0003**
Error	237	551			
Model 2 Females	***MS***	***DF***	***Eta²***	***F***	***P***
Country	927	1, 5	0.006	3.8	0.0511
Temperature	3815	1, 614	0.025	15.7	**< 0.0001**
PC1	5129	1, 614	0.033	21.1	**< 0.0001**
PC4	1506	1, 614	0.010	6.2	**0.0130**
Error	243	614			

796

Figure legends

Fig. 1 Larval time (a), pupal time (b), pupal mass (c), larval growth rate (d), adult body mass (e), abdomen mass (f), thorax mass (g), thorax-abdomen ratio (h), absolute fat content (i), relative fat content (j), wing length (k), wing area (l), wing loading (m), and aspect ratio (n) in *Lycaena tityrus* males (filled bars) and females (open bars) in relation to origin (country) and developmental temperature. Given are means ± 1 SE. In some cases the error bars are not visible. Group sample sizes range between 38 and 540 individuals. Different superscript letters above bars indicate significant differences among country by temperature groups (Tukey HSD after GLM).

Fig. 2 Exploratory behaviour (animals finding a food source within 90 minutes in %, a) and flight endurance in a vortex (b) in *Lycaena tityrus* males (filled bars) and females (open bars) in relation to origin (country) and developmental temperature. Given are means ± 1 SE. In some cases the error bars are not visible.

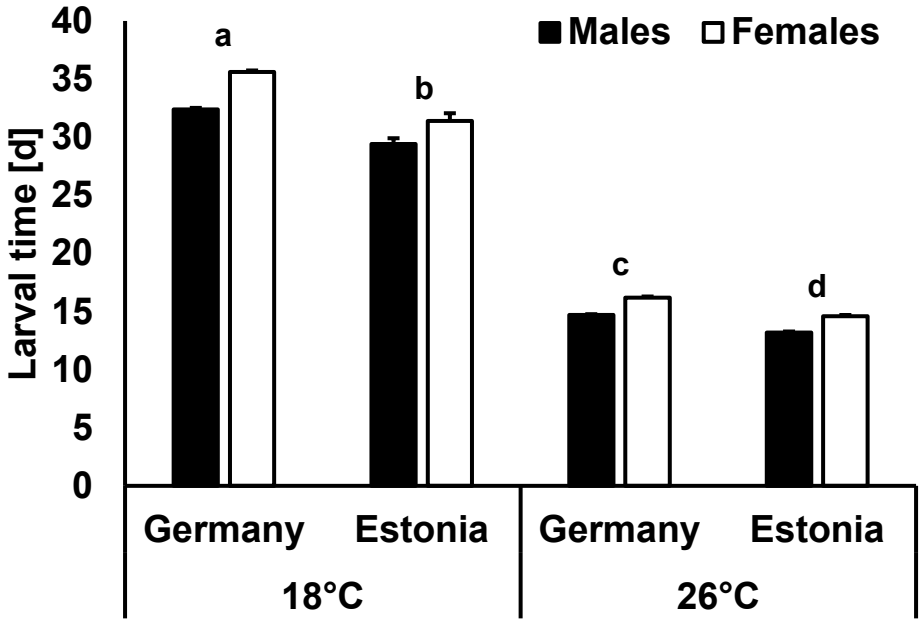

Males
Females
Larval time [d]
40
35
30
25
20
15
10
5
0
a
b
c
d
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1a

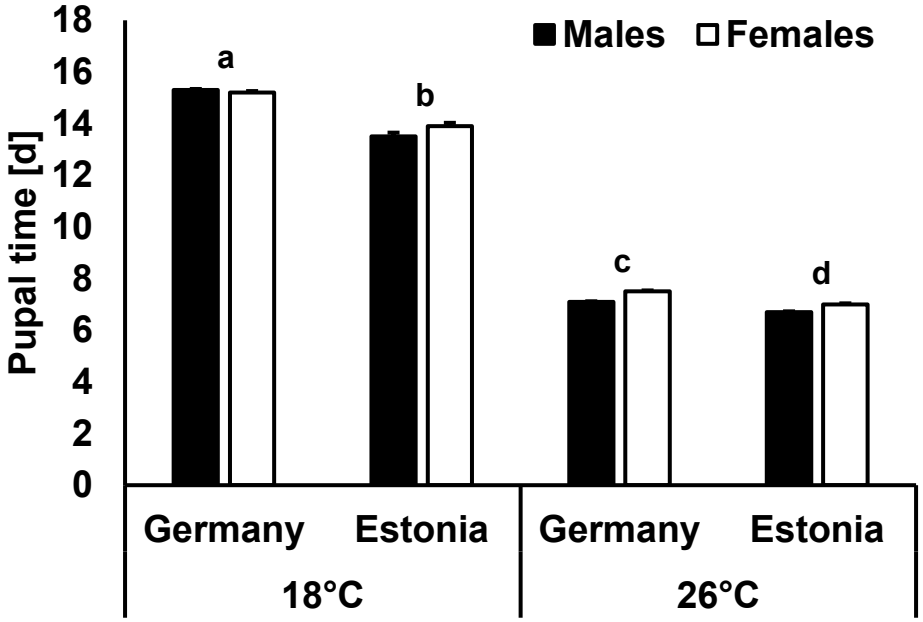
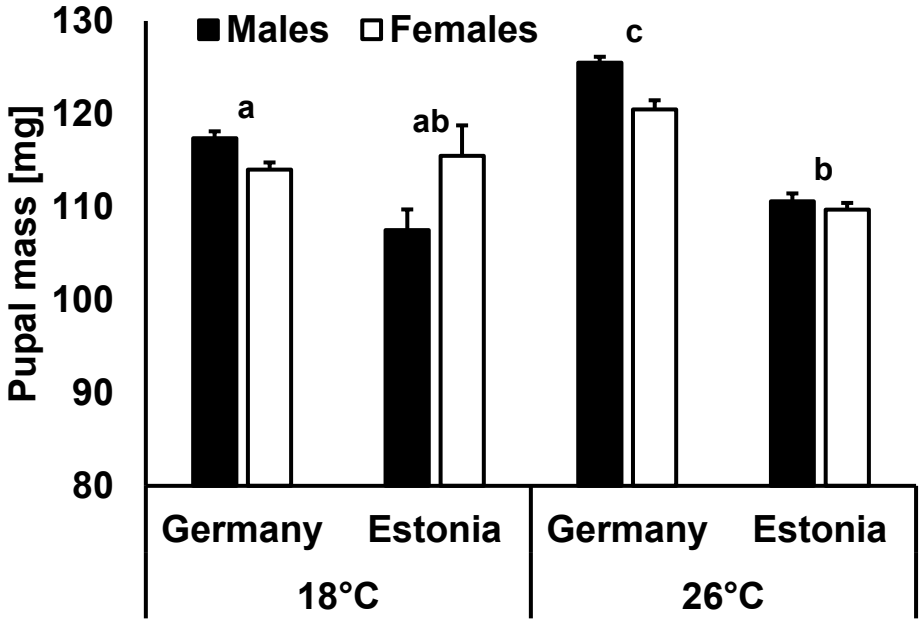

Males
Females
Pupal time [d]
18
16
14
12
10
8
6
4
2
0
a
b
c
d
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1b

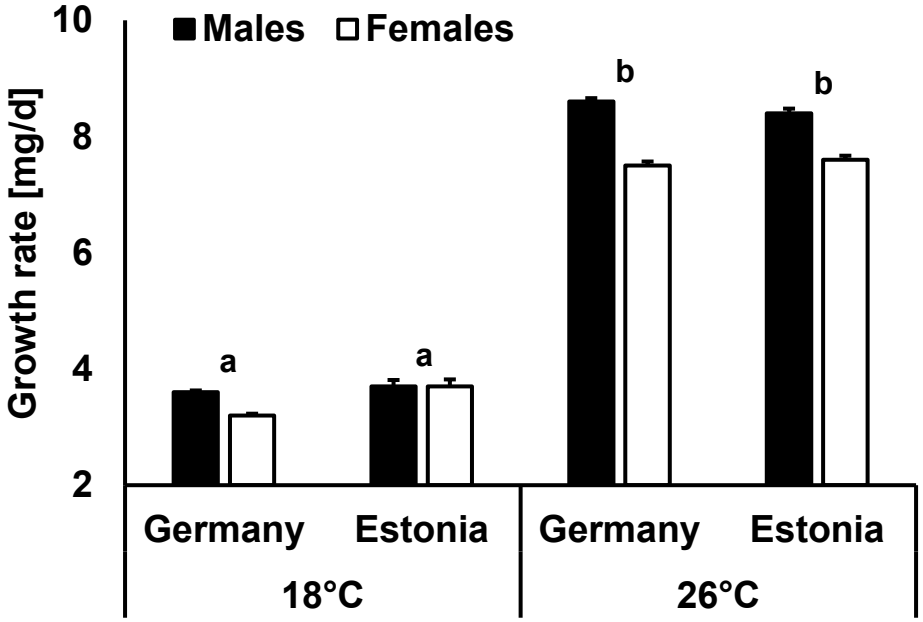
820



821

822 Fig. 1c

823

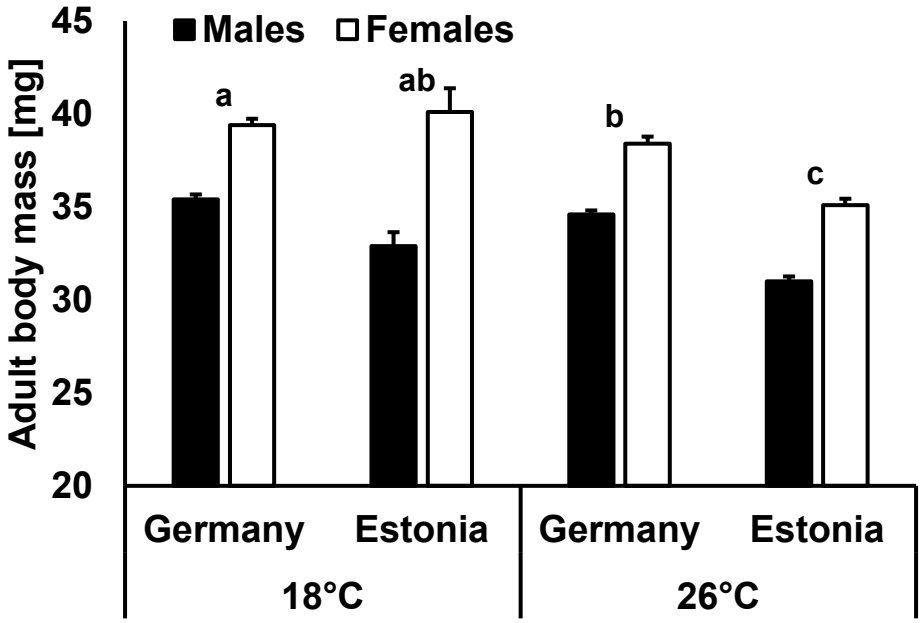


824

825 Fig. 1d

826

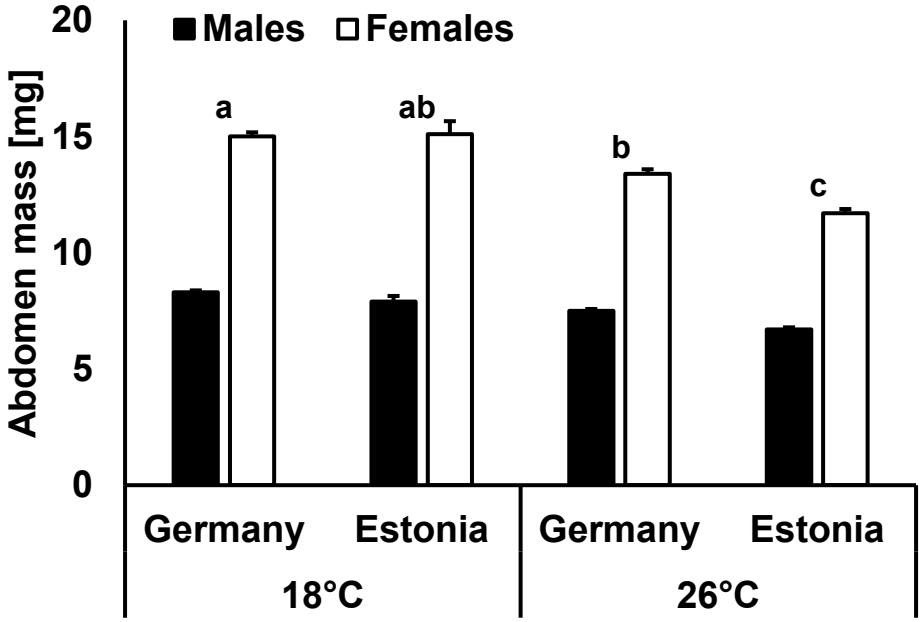
827



828

829 Fig. 1e

830



831

832 Fig. 1f

833

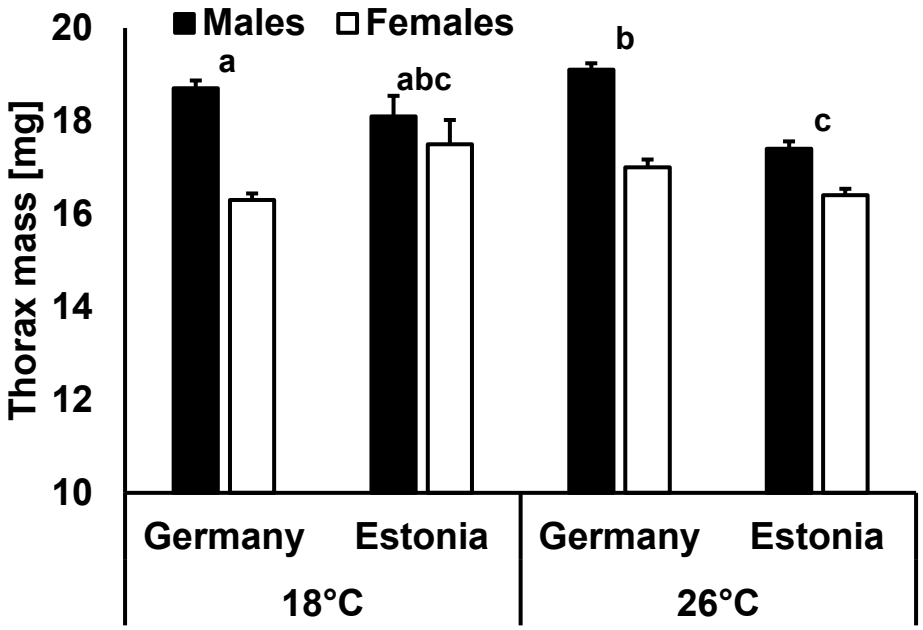

Males
Females
Thorax mass [mg]
20
18
16
14
12
10
a
abc
b
c
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1g

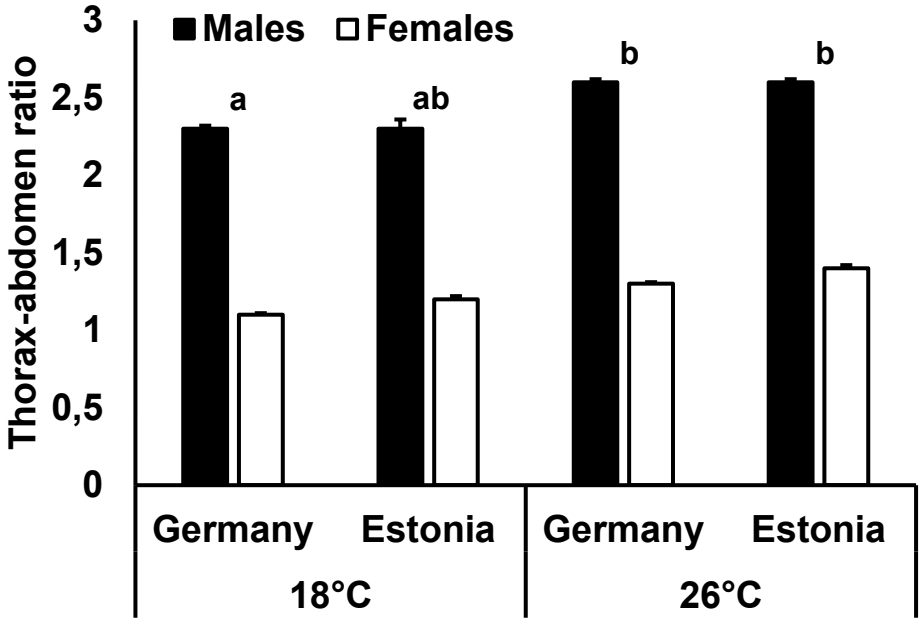

Males
Females
Thorax-abdomen ratio
3
2,5
2
1,5
1
0,5
0
a
ab
b
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1h

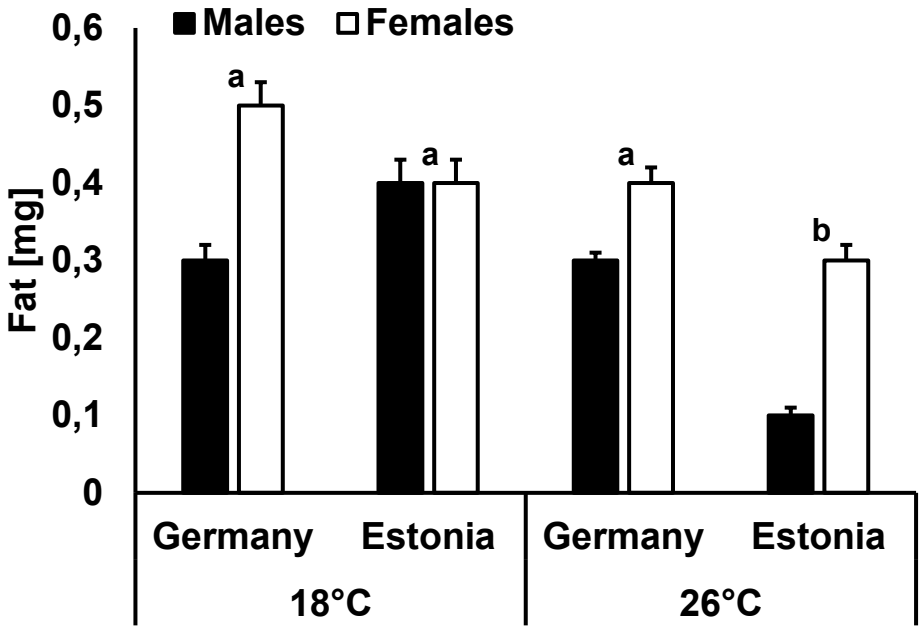

Males
Females
Fat [mg]
0,6
0,5
0,4
0,3
0,2
0,1
0
a
a
a
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1i

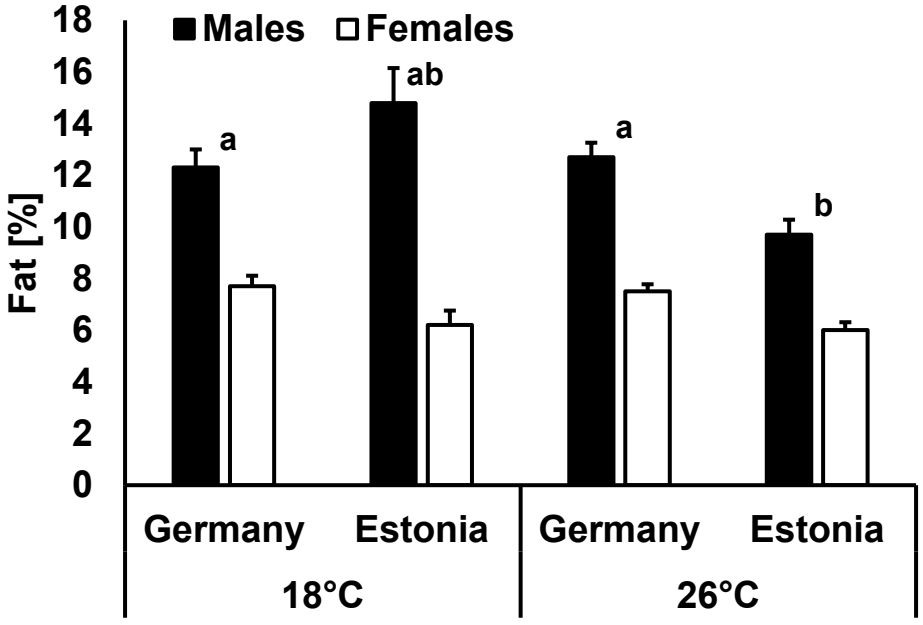

Males
Females
Fat [%]
18
16
14
12
10
8
6
4
2
0
a
ab
a
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1j

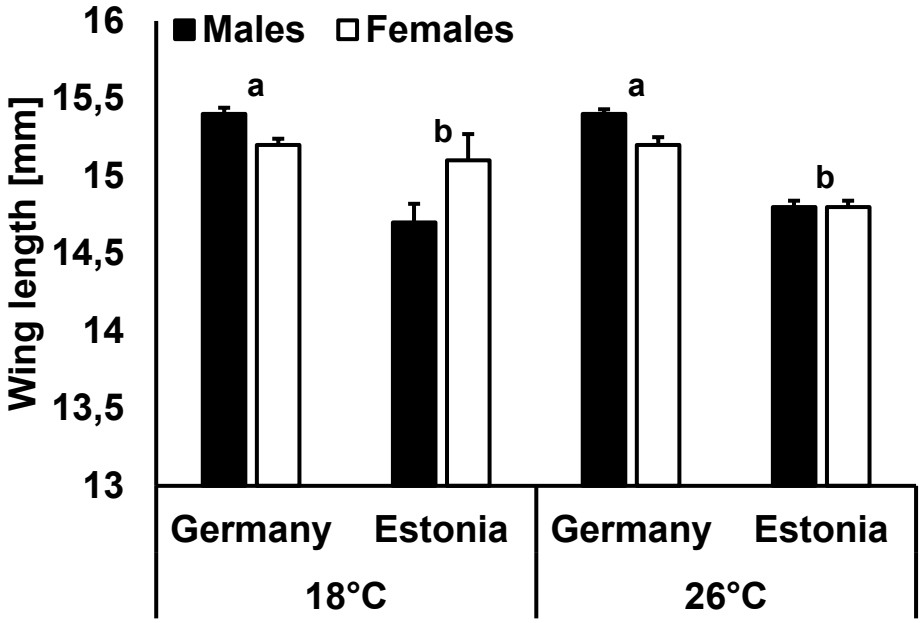

Males
Females
Wing length [mm]
16
15,5
15
14,5
14
13,5
13
a
b
a
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1k

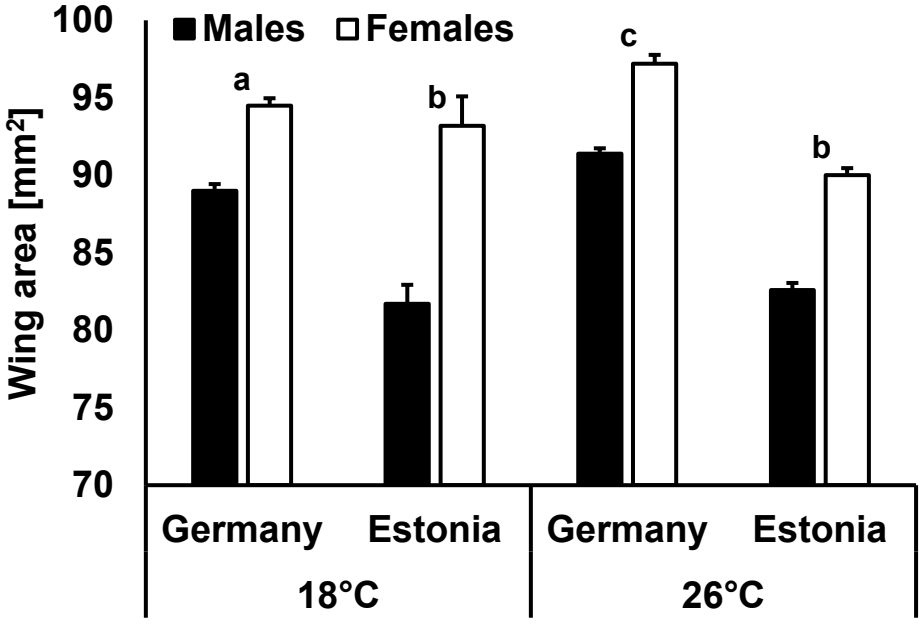

Males
Females
Wing area [mm²]
100
95
90
85
80
75
70
a
b
c
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1l

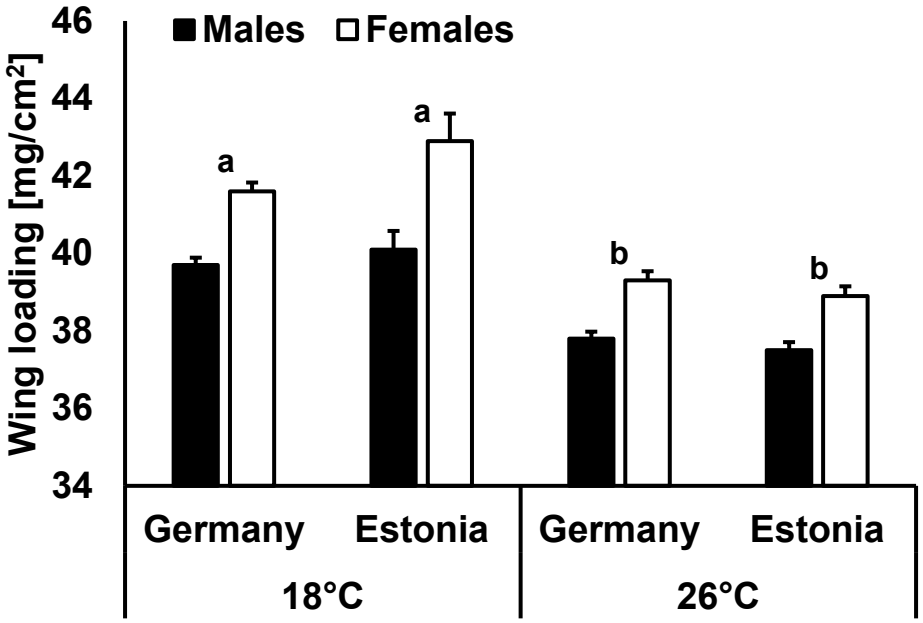

Males
Females
Wing loading [mg/cm²]
46
44
42
40
38
36
34
a
a
b
b
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1m

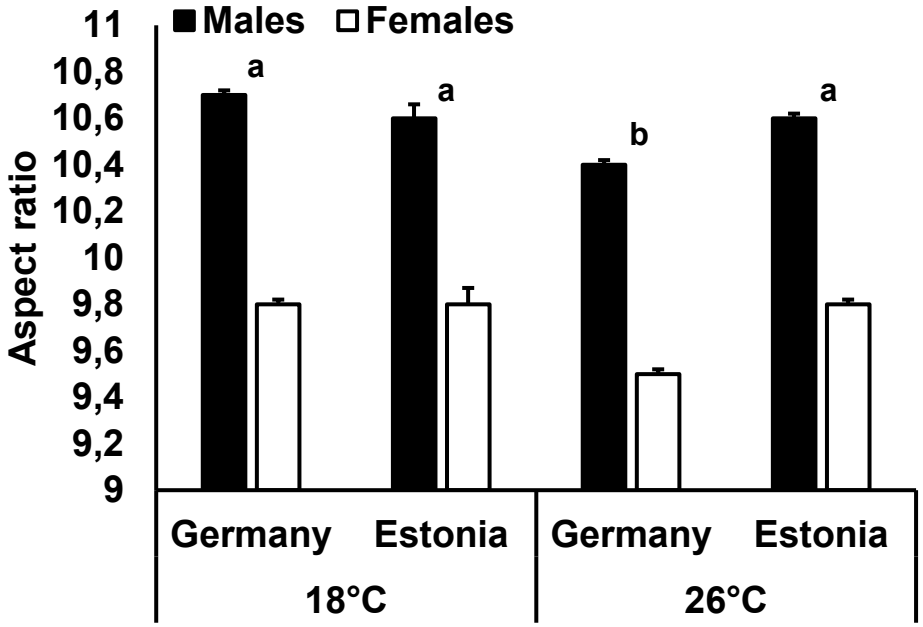
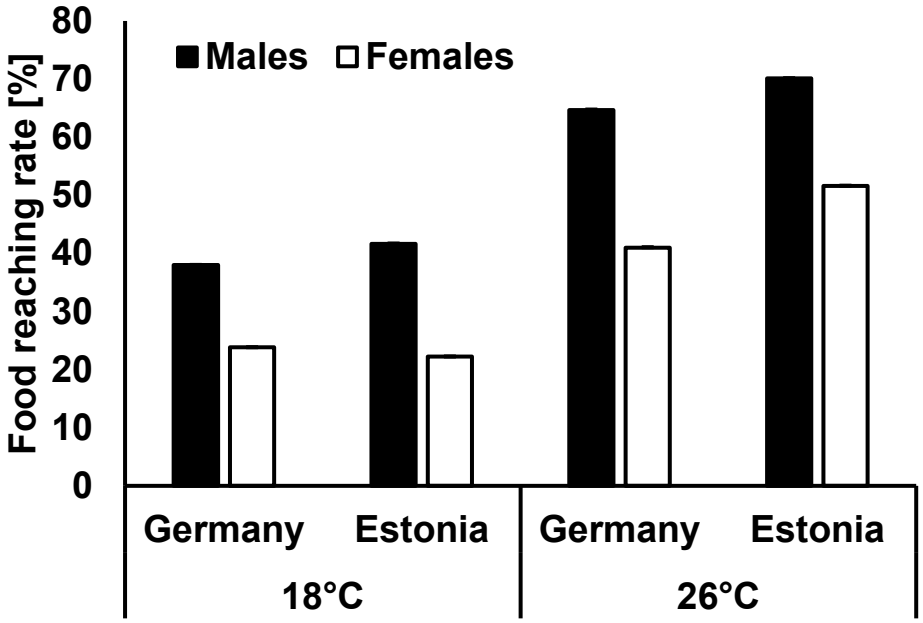

Males
Females
Aspect ratio
11
10,8
10,6
10,4
10,2
10
9,8
9,6
9,4
9,2
9
a
a
b
a
Germany
Estonia
Germany
Estonia
18°C
26°C

Fig. 1n

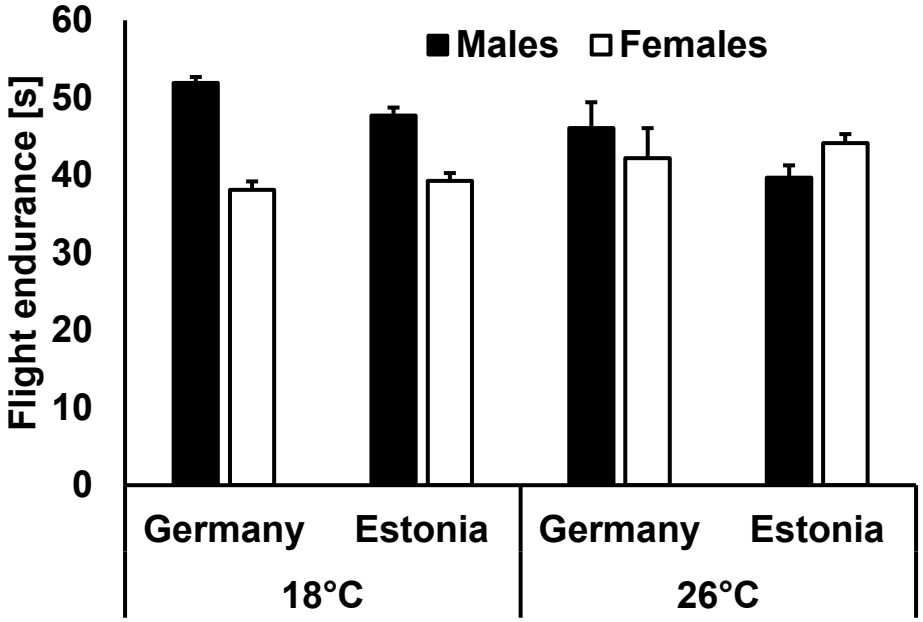
862



863

864 Fig. 2a

865



866

867 Fig. 2b

Appendices

A1: Tables 1 and 2 are exclusively based on the animals reared at 26°C, thus focusing on differences across countries.

Table 1 General linear mixed model results for the effects of country, sex (fixed factors), population (nested within country), and family (nested within population and country, random factors) on developmental and morphological traits in *Lycaena tityrus*. Significant *P*-values are given in bold. Models were constructed by a stepwise backwards elimination of non-significant factors.

Larval time	***MS***	***DF***	***F***	***P***
Country	440.0	1, 5	48.7	**0.0021**
Population (Country)	9.4	4, 936	6.0	**0.0021**
Sex	506.0	1, 936	325.1	**< 0.0001**
Error	1.6	936		
Pupal time	***MS***	***DF***	***F***	***P***
Country	57.2	1, 5	242.1	**< 0.0001**
Sex	22.4	1, 940	94.8	**< 0.0001**
Error	0.2	940		
Pupal mass	***MS***	***DF***	***F***	***P***
Country	32071	1, 5	19.3	**0.0113**
Population (Country)	1798	4, 829	6.1	**0.0002**
Family (Country*Pop)	317	106, 829	2.6	**< 0.0001**
Sex	1423	1, 829	11.6	**0.0007**
Country*Sex	2120	1, 829	17.4	**< 0.0001**
Error	122	829		

Table 1 continued

Growth rate	***MS***	***DF***	***F***	***P***
Country	2.2	1, 5	0.2	0.6775
Population (Country)	11.9	4, 829	6.2	**0.0002**
Family (Country*Pop)	2.1	106, 829	2.0	**< 0.0001**
Sex	186.6	1, 829	179.9	**< 0.0001**
Country*Sex	10.5	1, 829	10.2	**0.0015**
Error	1.0	829		
Adult body mass	***MS***	***DF***	***F***	***P***
Country	2075.8	1, 5	20.2	**0.0098**
Population (Country)	111.9	4, 729	3.8	**0.0059**
Family (Country*Pop)	31.6	106, 729	1.9	**< 0.0001**
Sex	2669.0	1, 729	162.4	**< 0.0001**
Error	16.4	729		
Abdomen mass	***MS***	***DF***	***F***	***P***
Country	245	1, 5	14.1	**0.0180**
Population (Country)	18.8	4, 729	3.4	**0.0119**
Family (Country*Pop)	5.9	106, 729	1.6	**0.0004**
Sex	5494.3	1, 729	1468.7	**< 0.0001**
Error	3.7	729		
Thorax mass	***MS***	***DF***	***F***	***P***
Country	237.1	1, 5	3.7	0.1246
Population (Country)	70.4	4, 728	8.9	**< 0.0001**
Family (Country*Pop)	8.5	106, 728	2.1	**< 0.0001**
Sex	455.1	1, 728	112.2	**< 0.0001**
Country*Sex	98.2	1, 728	24.2	**< 0.0001**
Error	4.1	728		

Table 1 continued

Thorax-abdomen ratio	***MS***	***DF***	***F***	***P***
Country	0.96	1, 5	2.9	0.1603
Population (Country)	0.36	4, 728	4.1	**0.0039**
Family (Country*Pop)	0.09	106, 728	1.4	**0.0069**
Sex	270.33	1, 728	4095.5	**< 0.0001**
Country*Sex	0.78	1, 728	11.8	**0.0006**
Error	0.07	728		
Fat (mg)	***MS***	***DF***	***F***	***P***
Country	2.22	1, 5	5.9	0.0694
Population (Country)	0.44	4, 693	5.0	**0.0009**
Family (Country*Pop)	0.10	106, 693	3.1	**< 0.0001**
Sex	2.78	1, 693	85.2	**< 0.0001**
Error	0.03	693		
Fat (%)	***MS***	***DF***	***F***	***P***
Country	682.9	1, 5	1.4	0.3006
Population (Country)	575.1	4, 694	5.6	**0.0004**
Family (Country*Pop)	117.8	106, 694	3.7	**< 0.0001**
Sex	3754.2	1, 694	118.4	**< 0.0001**
Error	31.7	694		
Wing length	***MS***	***DF***	***F***	***P***
Country	49.7	1, 5	45.4	**0.0019**
Population (Country)	1.2	4, 728	1.7	0.1451
Family (Country*Pop)	0.7	106, 728	2.9	**< 0.0001**
Sex	1.7	1, 728	6.5	**0.0110**
Country*Sex	5.5	1, 728	21.3	**< 0.0001**
Error	0.3	728		

Table 1 continued

Wing area	***MS***	***DF***	***F***	***P***
Country	11646	1, 5	68.7	**0.0009**
Population (Country)	183	4, 728	2.0	0.0925
Family (Country*Pop)	99	106, 728	2.9	**< 0.0001**
Sex	7958	1, 728	230.1	**< 0.0001**
Country*Sex	464	1, 728	13.4	**0.0003**
Error	35	728		
Wing loading	***MS***	***DF***	***F***	***P***
Country	14.0	1, 5	0.1	0.7497
Population (Country)	132.4	4, 729	8.4	**< 0.0001**
Family (Country*Pop)	17.0	106, 729	2.0	**< 0.0001**
Sex	342.2	1, 729	40.5	**< 0.0001**
Error	8.4	729		
Wing aspect ratio	***MS***	***DF***	***F***	***P***
Country	6.88	1, 5	80.9	**0.0001**
Population (Country)	0.08	4, 729	0.3	0.8515
Family (Country*Pop)	0.26	106, 729	3.1	**< 0.0001**
Sex	141.4	1, 729	1676.1	**< 0.0001**
Error	0.08	729		

Table 2 Results of a generalized linear model (exploratory behaviour, a) and of a general linear mixed model (flight performance in a vortex, b) for the effects of country, sex (both fixed), population (nested within country; random), and family (nested within population and country, random) in the butterfly *Lycaena tityrus* (models 1). Second models were constructed by additionally including PCs 1-4 and the results of the exploratory behaviour or performance test as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. The latter models were constructed separately for males and females, owing to strong variation in morphological traits among the sexes. Significant *P*-values are given in bold.

Table 2a

Model 1	***DF***	***Wald Chi²***	***P***
Country	1	4.7	**0.0295**
Sex	1	32.5	**< 0.0001**
Model 2 Males	***DF***	***Wald Chi²***	***P***
PC2	1	4.4	**0.0367**
Flight performance	1	10.0	**0.0016**
Model 2 Females	***DF***	***Wald Chi²***	***P***
PC2	1	8.8	**0.0030**

Table 2b

Model 1	***MS***	***DF***	***F***	***P***
Country	311	1, 5	0.3	0.6282
Population (Country)	1230	4, 723	2.7	**0.0353**
Family (Country*Pop)	495	106, 723	1.9	**< 0.0001**
Country*Sex	1322	1, 723	5.2	**0.0058**
Error	254	723		
Model 2 Males	***MS***	***DF***	***F***	***P***
Country	1659	1, 5	4.5	0.0623
Population (Country)	447	4, 222	1.2	0.3074
Family (Country*Pop)	431	106, 222	1.8	**0.0003**
PC1	7139	1, 222	29.5	**< 0.0001**
PC3	4593.8	1, 222	19.0	**< 0.0001**
Exploratory behaviour	1768	1, 222	7.3	**0.0074**
Error	242	222		
Model 2 Females	***MS***	***DF***	***F***	***P***
Country	923	1, 373	4.2	**0.0407**
PC1	3519	1, 373	16.1	**< 0.0001**
PC4	1153	1, 373	5.3	**0.0223**
Error	219	373		

A2: Tables 3 and 4 are exclusively based on the animals from Germany, thus focusing on differences across developmental temperature (18°C versus 26°C).

Table 3 General linear mixed model results for the effects of temperature, sex (fixed factors), population, and family (nested within population, both random factors) on developmental and morphological traits in *Lycaena tityrus*. Significant *P*-values are given in bold. Models were constructed by a stepwise backwards elimination of non-significant factors.

Larval time	***MS***	***DF***	***F***	***P***
Population	12.0	2, 981	1.4	0.2544
Family (Population)	10.4	59, 981	4.2	**< 0.0001**
Temperature	87024.0	1, 981	35069.0	**< 0.0001**
Sex	1440.0	1, 981	580.3	**< 0.0001**
Error	2.5	981		
Pupal time	***MS***	***DF***	***F***	***P***
Population	1.9	2, 980	2.7	**0.0765**
Family (Population)	0.8	59, 980	2.4	**< 0.0001**
Temperature	15805.2	1, 980	46050.4	**< 0.0001**
Sex	3.4	1, 980	9.8	**0.0018**
Temperature*Sex	8.9	1, 980	25.9	**< 0.0001**
Error	0.3	980		
Pupal mass	***MS***	***DF***	***F***	***P***
Population	2598	2, 981	6.4	**0.0027**
Family (Population)	484	59, 981	3.7	**< 0.0001**
Sex	5188	1, 981	39.6	**< 0.0001**
Temperature	15526	1, 981	118.4	**< 0.0001**
Error	131	981		

Table 3 continued

Growth rate	***MS***	***DF***	***F***	***P***
Population	14.1	2, 981	10.4	**0.0001**
Family (Population)	1.6	59, 981	2.4	**< 0.0001**
Sex	167.6	1, 981	258.5	**< 0.0001**
Temperature	5577.7	1, 981	8602.9	**< 0.0001**
Error	0.7	981		
Adult body mass	***MS***	***DF***	***F***	***P***
Population	97.5	2, 914	2.0	**0.1402**
Family (Population)	56.3	59, 914	2.9	**< 0.0001**
Sex	3116.2	1, 914	161.9	**< 0.0001**
Temperature	129.1	1, 914	6.7	**0.0098**
Error	19.3	914		
Abdomen mass	***MS***	***DF***	***F***	***P***
Population	0.11	2, 914	2.1	0.1361
Family (Population)	0.06	59, 914	1.9	**< 0.0001**
Sex	73.51	1, 914	2359.6	**< 0.0001**
Temperature	2.84	1, 914	91.2	**< 0.0001**
Error	0.03	914		
Thorax mass	***MS***	***DF***	***F***	***P***
Population	101.9	2, 914	7.7	**0.0009**
Family (Population)	15.5	59, 914	3.2	**< 0.0001**
Sex	1287.6	1, 914	264.0	**< 0.0001**
Temperature	96.3	1, 914	19.7	**< 0.0001**
Error	4.9	914		
Thorax-abdomen ratio	***MS***	***DF***	***F***	***P***
Population	0.28	2, 972	14.0	**< 0.0001**
Sex	119.6	1, 972	6023.0	**< 0.0001**
Temperature	5.2	1, 972	263.7	**< 0.0001**
Sex*Temperature	0.03	1, 972	1.6	0.2059
Error	0.02	972		

Table 3 continued

Fat (mg)	***MS***	***DF***	***F***	***P***
Population	23.1	2, 874	18.0	**< 0.0001**
Family (Population)	1.5	59, 874	3.1	**< 0.0001**
Sex	46.0	1, 874	93.9	**< 0.0001**
Error	0.5	874		
Fat (%)	***MS***	***DF***	***F***	***P***
Population	21.9	2, 874	18.7	**< 0.0001**
Family (Population)	1.4	59, 874	3.3	**< 0.0001**
Sex	49.2	1, 874	115.6	**< 0.0001**
Error	0.4	874		
Wing length	***MS***	***DF***	***F***	***P***
Population	1.4	2, 915	1.1	0.3416
Family (Population)	1.6	59, 915	5.3	**< 0.0001**
Sex	12.1	1, 915	40.0	**< 0.0001**
Error	0.3	915		
Wing area	***MS***	***DF***	***F***	***P***
Population	252	2, 914	1.5	0.2280
Family (Population)	202	59, 914	5.3	**< 0.0001**
Sex	6600	1, 914	171.4	**< 0.0001**
Temperature	1802	1, 914	46.8	**< 0.0001**
Error	39	914		
Wing loading	***MS***	***DF***	***F***	***P***
Population	150	2, 914	6.0	**0.0039**
Family (Population)	29	59, 914	3.2	**< 0.0001**
Sex	586	1, 914	64.6	**< 0.0001**
Temperature	910	1, 914	100.3	**< 0.0001**
Error	9	914		

Table 3 continued

Aspect ratio	***MS***	***DF***	***F***	***P***
Population	0.03	2, 914	0	0.9311
Family (Population)	0.48	59, 914	5	**< 0.0001**
Sex	176.41	1, 914	1877	**< 0.0001**
Temperature	12.76	1, 914	136	**< 0.0001**
Error	0.09	914		

Table 4 Results of a generalized linear model (exploratory behaviour, a) and of a general linear mixed model (flight performance in a vortex, b) for the effects of temperature, sex (both fixed), population (random), and family (nested within population, random) in the butterfly *Lycaena tityrus* (models 1). Second models were constructed by additionally including PCs 1-4 and the results of the exploratory behaviour or performance test as covariates. All models were constructed by a stepwise backwards elimination of non-significant factors. The latter models were constructed separately for males and females, owing to strong variation in morphological traits among the sexes. Significant *P*-values are given in bold.

Table 4a

Model 1	***DF***	***Wald Chi²***	***P***
Population	2	6.1	**0.0465**
Temperature	1	43.2	**< 0.0001**
Sex	1	32.0	**< 0.0001**
Model 2 Males	***DF***	***Wald Chi²***	***P***
Temperature	1	24.1	**< 0.0001**
PC1	1	10.2	**0.0014**
PC4	1	4.3	**0.0391**
Flight performance	1	7.4	**0.0066**
Model 2 Females	***DF***	***Wald Chi²***	***P***
Temperature	1	13.2	**0.0003**

Table 4b

Model 1	***MS***	***DF***	***F***	***P***
Population	1005	2, 909	2.6	0.0781
Family (Population)	426	59, 909	1.9	**< 0.0001**
Sex*Temperature	2951	1, 909	13.3	**< 0.0001**
Sex	18770	1, 909	84.5	**< 0.0001**
Error	222	909		
Model 2 Males	***MS***	***DF***	***F***	***P***
Population	1222	2, 410	6.5	**0.0017**
Temperature	4766	1, 410	25.1	**< 0.0001**
PC1	1941	1, 410	10.2	**0.0015**
PC3	7442	1, 410	39.3	**< 0.0001**
Flower test	1585	1, 410	8.4	**0.0040**
Error	190	410		
Model 2 Females	***MS***	***DF***	***F***	***P***
Population	408	2, 373	1.0	0.3773
Family (Population)	473	59, 373	2.2	**< 0.0001**
PC1	2914	1, 373	13.5	**0.0003**
PC3	4356	1, 373	20.1	**< 0.0001**
PC4	942	1, 373	4.4	**0.0376**
Error	216	373		

A3: Table 5 shows trait variation across replicate populations.

Table 5 Developmental, morphological, and physiological traits in relation to population (country) in *Lycaena tityrus*. Given are means ± 1 SE. Group sample sizes range between 60 and 399 individuals. Different superscript letters within a row indicate significant differences among groups (Tukey HSD).

	Germany			Estonia		
Trait	**Guest**	**Ueckermünde**	**Waren**	**Mahu**	**Kasispea**	**Karepa**
Larval time (days)	23.9 ± 0.56^{a}	24.6 ± 0.53^{a}	24.3 ± 0.46^{a}	15.2 ± 0.37^{b}	15.4 ± 0.42^{b}	15.9 ± 0.47^{b}
Pupal time (days)	10.9 ± 0.22^{a}	11.2 ± 0.22^{a}	11.1 ± 0.20^{a}	7.5 ± 0.15^{b}	7.4 ± 0.18^{b}	7.5 ± 020^{b}
Pupal mass (mg)	119.7 ± 0.77^{ab}	122.4 ± 0.81^{a}	117.5 ± 0.59^{bd}	109.5 ± 0.81^{c}	114.2 ± 1.25^{cd}	109.7 ± 1.01^{c}
Growth rate (mg/day)	5.9 ± 0.16^{a}	6.0 ± 0.15^{a}	5.7 ± 0.11^{a}	7.7 ± 0.13^{b}	7.7 ± 0.15^{b}	7.3 ± 0.14^{b}
Adult body mass (mg)	37.4 ± 0.33^{a}	37.1 ± 0.29^{ab}	36.1 ± 0.24^{bc}	33.0 ± 0.34^{d}	36.1 ± 0.56^{ac}	33.0 ± 0.46^{d}
Abdomen mass (mg)	11.5 ± 0.27^{a}	10.7 ± 0.21^{ab}	10.4 ± 0.18^{bce}	9.1 ± 0.24^{d}	11.6 ± 0.41^{ac}	9.4 ± 0.30^{de}
Thorax mass (mg)	17.7 ± 0.15^{a}	18.5 ± 0.16^{b}	17.5 ± 0.13^{ac}	16.9 ± 0.16^{c}	17.4 ± 0.22^{ac}	16.8 ± 0.20^{c}
Thorax-abdomen ratio	1.8 ± 0.04^{ac}	1.9 ± 0.04^{b}	1.9 ± 0.03^{abc}	2.0 ± 0.05^{b}	1.6 ± 0.07^{c}	2.0 ± 0.06^{ab}
Fat (mg)	0.5 ± 0.03^{a}	0.3 ± 0.01^{b}	0.3 ± 0.01^{c}	0.3 ± 0.02^{b}	0.2 ± 0.02^{bc}	0.2 ± 0.01^{b}
Fat (%)	14.0 ± 0.76^{a}	7.4 ± 0.30^{bd}	10.0 ± 0.35^{c}	9.2 ± 0.52^{bc}	5.3 ± 0.46^{d}	7.7 ± 0.46^{bd}
Wing length (mm)	15.2 ± 0.03^{a}	15.4 ± 0.04^{b}	15.3 ± 0.03^{ab}	14.9 ± 0.04^{c}	14.9 ± 0.06^{c}	14.8 ± 0.05^{c}
Wing area (mm^2)	91.7 ± 0.46^{ad}	93.7 ± 0.46^{b}	92.8 ± 0.36^{ab}	87.1 ± 0.52^{cd}	89.7 ± 0.79^{d}	85.9 ± 0.66^{c}
Wing loading (mg/cm^2)	40.7 ± 0.23^{a}	39.4 ± 0.20^{b}	38.9 ± 0.17^{bd}	37.8 ± 0.24^{c}	40.2 ± 0.37^{ab}	38.3 ± 0.31^{cd}
Wing aspect ratio	10.1 ± 0.04^{ab}	10.1 ± 0.03^{ab}	10.1 ± 0.03^{ab}	10.2 ± 0.04^{a}	9.9 ± 0.05^{b}	10.2 ± 0.05^{a}

EIGENSTÄNDIGKEITSERKLÄRUNG

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Unterschrift des Promovenden